THE ROLE OF ETHANOL AND NEUROANATOMICAL REGIONS IN SENSITIVITY TO DELAY AND SENSITIVITY TO MAGNITUDE OF REWARD

By

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Abstract.

Relative preference for smaller, sooner rewards over larger, later rewards ("delay discounting") is increased by acute ethanol. However, it is unknown whether this effect is driven by a difference in sensitivity to the reinforcer delay or a difference in sensitivity to the reinforcer magnitude, because typical delay discounting tasks manipulate both parameters simultaneously. Additionally, it is unknown which brain regions may play a role in these different aspects of delay discounting.

To investigate this, two studies were conducted in separate groups of male Long Evans rats. The first examined the effects of acute systemic administration of ethanol on performance in two separate tasks that measured sensitivity to delay and sensitivity to magnitude. The second examined the effects of acute temporary inactivation of the nucleus accumbens core (AcbC) and lateral orbitofrontal cortex (lOFC) on two separate tasks that measured sensitivity to delay and sensitivity to magnitude as well as a delay discounting task.

None of the acute manipulations had any effect on the measures of sensitivity to delay or sensitivity to magnitude, in contrast to their effects on delay discounting in previous literature. Nonetheless, there were several additional findings of interest. First, rats with high sensitivity to delay were found to be resistant to the behaviorally suppressant effects of a moderate dose of ethanol (0.9 g/kg). Second, inactivation of the AcbC was found to significantly decrease delay discounting. Furthermore, this effect of AcbC inactivation was found to be most prominent in animals with initially low levels of delay discounting.

In summary, these results suggest a fundamental difference between the tasks used to separately measure sensitivity to delay and magnitude and those traditionally used to measure delay discounting. Additionally, the findings of a possible link between sensitivity to delay and the suppressant effects of ethanol, as well as a novel role for the AcbC in delay discounting, provide a springboard for future research.

Chapter 1. Introduction

Alcohol, known primarily in the scientific literature as ethyl alcohol or ethanol, has been consumed for millennia. Chemical analysis of pottery shards from the Henan province of China suggests that it was produced as early as 9000 years ago (McGovern et al., 2004). This practice was not unique to China, and several other cultures took up the production of ethanol as well (Hanson, 1995). In the time since its inception, ethanol has been used for nutrition, medicine, and ceremony (Hanson, 1995). However, one of the primary reasons for the consumption of ethanol is for its psychoactive effects. At low doses (~0.05 - 0.10% blood ethanol concentration [BEC]), ethanol can increase euphoria, sociability, and relaxation (Pohorecky & Brick, 1988). Given these positive effects, it is not surprising that, according to a survey taken by The Behavioral Risk Factor Surveillance System (Centers for Disease Control, 2010), over 50% of the adult population of the United States had consumed ethanol within the past 30 days.

Consumption of ethanol can have negative consequences. The Centers for Disease Control (2013) reported that ethanol was the cause of an average of 87,798 deaths per year from 2006 - 2010. Roughly half of these deaths were attributable to an acute dose of ethanol, while the rest were due to the effects of chronic ethanol use. Often, these deaths occurred in individuals who suffered from an alcohol use disorder, which is highly prevalent in the United States. Indeed, according to the National Institute on Alcohol Abuse and Alcoholism (NIAAA), approximately 18 million people in the United States suffer from alcohol use disorder (Hasin et al., 2007). Alcohol use disorder

is explicitly defined by the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (American Psychiatric Association, 2013) as:

"A problematic pattern of alcohol use leading to clinically significant impairment or distress, as manifested by at least two of the following, occurring within a 12-month period:

- Alcohol is often taken in larger amounts or over a longer period than was intended.
- There is a persistent desire or unsuccessful efforts to cut down or control alcohol use.
- A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects.
- Craving, or a strong desire or urge to use alcohol.
- Recurrent alcohol use resulting in a failure to fulfill major role obligations at work, school, or home.
- Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of alcohol.
- Important social, occupational, or recreational activities are given up or reduced because of alcohol use.
- Recurrent alcohol use in situations in which it is physically hazardous.
- Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol.

- Tolerance, as defined by either of the following:
  - A need for markedly increased amounts of alcohol to achieve intoxication or desired effect.
  - A markedly diminished effect with continued use of the same amount of alcohol.
- Withdrawal, as manifested by either of the following:
  - The characteristic withdrawal syndrome for alcohol.
  - Alcohol (or a closely related substance, such as a benzodiazepine) is taken to relieve or avoid withdrawal symptoms."

It is apparent from this definition that those suffering from alcohol use disorder do not simply have an increased risk of mortality, but also have a greatly decreased quality of life. Additionally, alcohol use disorder has a high degree of comorbidity with psychiatric disorders such as anxiety disorders, depression, conduct disorder, as well as other substance abuse disorders (Kessler et al., 1997). Unsurprisingly, there is a strong impetus to understand the exact nature of this disorder to ameliorate its symptoms.

A better understanding of alcohol use disorder can come from several different research foci, for example, describing the effects of chronic and acute ethanol on the central nervous system and on behavior, identifying behavioral and physiological biomarkers that can predict a susceptibility to alcohol use disorder, and delineating the genetic influences contributing to these relationships. Of course, many studies examine all of these components together. For the purposes of the current research, however, the primary focus is on *behaviors* associated with alcohol use disorder and ethanol consumption.

Several behaviors are associated with alcohol use disorder. For example, individuals with alcohol use disorder have heightened novelty-seeking (Kampov-Polevoy et al., 2004), a stronger hedonic response to sweet taste (Kampoy-Polevoy et al., 1997), performance deficits in complex, but not simple, working memory tasks (Rapeli et al., 1997), decreased executive function in tasks assessing planning and cognitive flexibility (Noël et al., 2001), altered behavioral responses to ethanol-related cues (Cox et al., 1999), and increased impulsivity (Dick et al., 2010). Because impulsivity is the primary focus of this dissertation, I will discuss it in more detail below.

Impulsivity.

Impulsivity has been repeatedly associated with alcohol use disorder, as well as substance abuse in general (Crews & Boettiger, 2009; de Wit, 2009; Dick et al., 2010; Lejuez et al., 2010). Impulsivity has been variously defined as "taking actions that appear poorly conceived, prematurely expressed, unduly risky, or inappropriate to the situation and that often result in undesirable consequences", "the tendency to engage in inappropriate or maladaptive behaviors", "and doing things or tending to do things suddenly and without careful thought" (Daruna & Barnes, 1993, p. 23; de Wit, 2009; Merriam-Webster Online, 2013). Given these definitions, one can see how heightened impulsivity could relate to diagnostic behaviors inherent in alcohol use disorder, such as "alcohol is often taken in larger amounts or over a longer period than was intended", "there is a persistent desire or unsuccessful efforts to cut down or control alcohol use", "recurrent alcohol use results in a failure to fulfill major role obligations at work, school, or home", and "recurrent alcohol use in situations in which it is physically hazardous" (American Psychiatric Association, 2013).

Multiple different measures of impulsivity have been devised, and many of these are thought to underlie distinct core processes that only partially overlap (Evenden, 1999). Thus, impulsivity itself is a term that represents several different behavioral patterns that are each the product of various neurocognitive processes.

Broadly speaking, there are two methods for determining an individual's level of impulsivity: self-report about behaviors people engage in when placed in a variety of situations and procedural methods that examine the actual behaviors that people engage in during a specific assessment session. Both methods of assessment have led to two distinct nomenclatures that only partially overlap. The two methods can yield different results, and often, self-report of a facet of impulsivity is not correlated with performance in a task that purports to measure that same facet of impulsivity (for example, self-report of motor impulsivity did not correlate with performance in the stop or go/no-go task, Reynolds et al., 2006a). Because it is impossible to use self-report measures in animal studies, I will be focusing on procedural methods of impulsivity in this dissertation. Nonetheless, it is important to keep these two different methods in mind when reviewing human literature, especially considering the fact that they likely measure distinct processes.

Within the procedural literature, research has primarily focused on two distinct types of impulsivity: behavioral disinhibition and delay discounting. It should be noted that there are other forms of impulsivity, such as reflection impulsivity, wherein an individual makes a decision before they have all the information pertinent to that decision (see Evenden, 1999, for a comprehensive list). Additionally, several behaviors, such as novelty seeking and risk taking, are conceptually similar to impulsivity in that they ignore

the potential for bad outcomes and are associated with psychopathologies (Kampov-Polevoy et al., 2004; Kreek et al., 2005). Nonetheless, for the duration of this dissertation, I will primarily be discussing behavioral disinhibition and delay discounting.

Behavioral disinhibition can be described as the inability to inhibit an action that is in some way inappropriate or undesirable. For example, an individual may normally refrain from making sharp remarks, but after a few drinks, the same individual may become *disinhibited* and begin to insult people without provocation. In tasks that measure this process, an increase in behavioral disinhibition is typically measured as an increase in inappropriate responses occurring prior to any eliciting stimulus or an increase in the lead-time required to suppress such responses.

On the other hand, delay discounting describes the process by which the objective value of an item that is available following a delay is reduced. Delay discounting is typically described in terms of a choice between a small, sooner reward and a large, later reward (see Fig. 1a). Choice of the smaller reward is thought to be more impulsive and to represent a relative inability to delay gratification (Ainslie, 1975; Logue, 1988; Mischel, 1981). For example, an individual may prefer 5 dollars now as opposed to waiting for 20 dollars in a week. In tasks that measure this process, an increase in delay discounting is typically measured as an increase in the number of choices of the small, sooner reward (Evenden & Ryan, 1996; Mazur, 1987; Richards et al., 1997).

Ethanol and Impulsivity.

Impulsivity is heightened in chronic users of a number of different drugs, including heroin (Kirby et al., 1997), cocaine (Coffey et al., 2003), methamphetamine (Hoffman et al., 2006), and nicotine (Mitchell, 1999; see Jentsch & Taylor, 1999; Perry

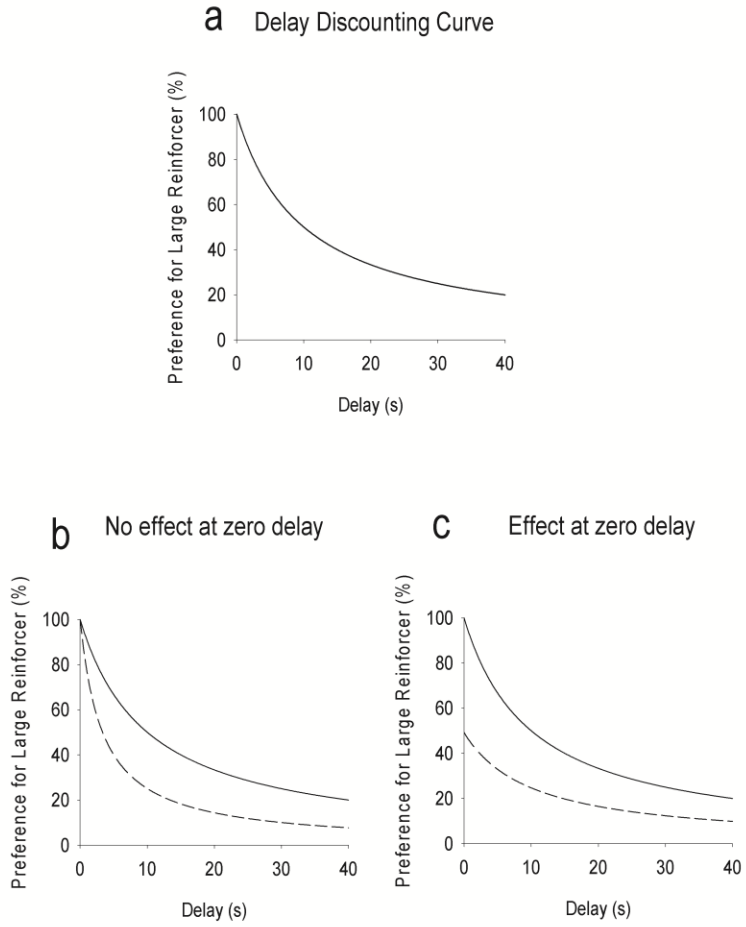


Figure 1. Different means by which delay discounting can be altered. **a)** A hypothetical delay discounting curve. The relative preference for the larger, delayed reward over the smaller, immediate reward decreases as a function of increasing delay to the larger reward. **b)** In this example, the dashed line represents an increase in delay discounting that *does not* impact the value of the large reward at zero delay (0 s). **c)** In this example, the dashed line represents an increase in delay discounting that *does* impact the value of the large reward at zero delay (0 s).

& Carroll, 2008; and Winstanley et al., 2010 for reviews of the literature regarding drugs of abuse and impulsivity). Additionally, some drugs of abuse can themselves acutely alter impulsivity, including cocaine (Fillmore et al., 2002) and amphetamine (de Wit et al., 2002). It is therefore not necessarily surprising that impulsivity is also heightened in individuals suffering from alcohol use disorder, and that ethanol itself can increase impulsivity (Dick et al., 2010).

When examining specific elements of impulsivity, individuals with alcohol use disorder generally have both heightened behavioral disinhibition and increased delay discounting. For example, a study by Bjork et al. (2004) found that detoxified alcohol-dependent patients had more inhibitory failures in a task designed to measure behavioral disinhibition than control subjects. The same study also found that alcohol-dependent subjects preferred the smaller, sooner reward much more in a delay discounting task than control subjects. Although studies examining behavioral disinhibition have yielded mixed results (Finn et al., 2002, Kamarajan et al., 2005; Rodríguez-Jiménez, 2006), by and large the majority of studies have found that individuals with alcohol use disorder have increased delay discounting (Bobova et al., 2009; Claus et al., 2011; MacKillop et al., 2010; Petry, 2001; Vuchinich & Simpson, 1998).

While these studies do suggest a relationship between heightened impulsivity and alcoholism, their design precludes causal links to be inferred. It may be that steeper delay discounting underlies a higher propensity to become an alcoholic, or that chronic ethanol increases delay discounting, or it may that both of these are true (see Mitchell, 2004; Perry & Carroll, 2008; for full lists of the potential underlying causal models). In humans, a direct test of the nature of these causal links requires difficult and time-

consuming longitudinal studies, and such studies cannot easily determine the underlying mechanism (e.g. which brain region, gene, etc. is necessary). These problems can be partially overcome through the use of animal models.

Research assessing the relationship between ethanol consumption and impulsivity in animals has yielded mixed results. Investigators have examined the link between ethanol-naïve levels of impulsivity and the consumption of ethanol in inbred animals, animals selectively bred for high or low levels of ethanol consumption, and outbred animals. Studies have shown that inbred C57BL/6J mice, which drink larger quantities of ethanol compared to another inbred line of mice known as DBA/2J mice (Belknap et al., 1993), also have greater behavioral disinhibition than DBA/2J mice (Loos et al., 2010b; Patel et al., 2006; Moschak et al., 2013). However, mice that were selectively bred for high ethanol consumption (HDID lines) did not show such heightened behavioral disinhibition (Tipps et al., 2014). With regards to delay discounting, studies using lines of selectively bred mice and rats have shown that some, but not all, lines selectively bred for higher ethanol consumption have steeper discounting (relationship: Oberlin & Graham, 2009; Wilhelm & Mitchell, 2008; no relationship: Wilhelm et al., 2007; Wilhelm & Mitchell, 2012). Research with outbred rats is equally ambiguous: One study found a direct link between baseline levels of discounting and ethanol consumption (Poulos et al., 1995), while another did not (Diergaarde et al., 2012). It should be noted that the daily amount of ethanol consumed by the high drinking animals in many of these studies was similar to the amount consumed by heavy drinkers (according the definition used by the NIAAA, and using dose conversions between species provided by Reagan-

Shaw et al., 2008) and can lead to behavioral effects upon acute oral administration (Lê & Israel, 1994).

Much research has also examined the effect of acute ethanol on impulsivity. Ethanol increases behavioral disinhibition in humans (Abroms et al., 2003; de Wit et al., 2000; Fillmore & Vogel-Sprott, 1999; Marczinski et al., 2003; Ramaekers et al., 2006). However, the delay discounting literature is less compelling, as the majority of studies investigating the effect of acute ethanol on delay discounting in healthy adults found no effect (Bidwell et al., 2013; Caswell et al., 2013; Dougherty et al., 2008; Ortner et al., 2003; Richards et al., 1999; but see Altamirano et al., 2011). Nonetheless, it is important to consider the nature of the studies used. Reynolds et al. (2006b) found that, with short delays and real-time monetary payout, ethanol *did* increase delay discounting in human subjects. Reynolds et al. (2006b) used this design in part to make more accurate comparisons to animal models of discounting because ethanol *has* been shown to increase delay discounting in animals (Evenden & Ryan, 1999; Hellemans et al., 2005; Olmstead et al., 2006; Poulos et al., 1998; Tomie et al., 1998; but see Wilhelm & Mitchell, 2012).

The acute effects of ethanol on behavioral inhibition have been tested in several studies using rodents. Notably, the species of rodent used seems to determine the effect of ethanol. Studies using mice have predominantly shown that ethanol increases behavioral disinhibition (Moschak et al., 2013; Oliver et al., 2009; Olmstead et al., 2009; Tipps et al., 2014), while studies using rats have found no effect (Bizarro et al., 2003; Hellemans et al., 2005; Moschak & Mitchell, 2012).

Sensitivity to Delay and Magnitude.

While ethanol has been studied within the context of both behavioral disinhibition and delay discounting, for the remainder of this dissertation I will focus on delay discounting. As mentioned above, several studies have shown that ethanol increases delay discounting in rats (Evenden & Ryan, 1999; Hellemans et al., 2005; Olmstead et al., 2006; Poulos et al., 1998; Tomie et al., 1998; but see Wilhelm & Mitchell, 2012). The typical interpretation of these studies is that ethanol in some way altered sensitivity to the delay (see Fig. 1b). For example, ethanol may have made the delay seem more aversive or longer, or may have caused the individual to ignore the delayed option entirely (regardless of its length) in favor of the immediate reward. Such interpretations are consistent with the notion that the delay discounting task measures the ability to *delay* gratification (Ainslie, 1975).

However, these interpretations do not fit well with the actual data. In two of the studies examining ethanol's effects on delay discounting, ethanol increased choice of the immediate small reward even when the large reward was not delayed (Evenden & Ryan, 1999; Tomie et al., 1998; most of the other studies had no condition wherein the large reward was not delayed). In other words, when the animals were faced with the choice between a small, immediate reward and a large, immediate reward, ethanol decreased their choice of the large, immediate reward (see Fig. 1c; intercept when delay = 0 s). Because there was no delay present during this choice, it is difficult to see how ethanol could have altered delay sensitivity.

There are a few possible explanations for these data. One is that ethanol *does* alter delay sensitivity, but in the sense that it results in expression of a conditioned aversion towards the location associated with the delayed reward, even on trials when that delay is

0 seconds (s). That is, in the delay discounting tasks used in Evenden & Ryan (1999) and Tomie et al. (1998), the delay to the large reward was initially set to zero at the beginning of each session, requiring subjects to choose between the small, immediate reward and the large, immediate reward. The delay to the large reward was thereafter progressively increased throughout the session. Thus, the animal had a choice between two levers: one *always* associated with an immediate reward, and the other *usually* associated with a delayed reward. The animal may have simply generalized one lever as "immediate" and the other as "delayed", even though the second was not *always* delayed (see a full discussion of this type of stimulus aversion in Wilhelm & Mitchell, 2010). Thus ethanol could have decreased choice of the "delayed" lever, regardless of whether a delay was actually associated with that lever at any given moment in the session. If true, this would support the previous interpretations that ethanol alters some aspect of delay sensitivity (as illustrated by the left side of Fig. 2a).

Another possibility is that ethanol does *not* alter delay sensitivity, but instead alters the ability of animals to process the magnitude and/or utility of the reward. This could occur in two ways. First, if the animal is unable to accurately discriminate between the sizes of the two rewards, the animal may exhibit relative indifference between the two. This in turn could manifest as a decreased preference for the large reward, regardless of whether it is delayed or not. Second, size discrimination could be intact, but the relative subjective value of the large reward compared to the small reward could be diminished (i.e. the diminishing marginal utility may be altered, see Appendix 1 for a more detailed consideration of this idea). If either is true, this would support an

alternative interpretation that ethanol alters some form of magnitude sensitivity (as illustrated by the right side of Fig. 2a).

Determining the nature of ethanol's effects on delay discounting, whether it be by altering delay or magnitude sensitivity, is important for a number of reasons. First, ethanol's mechanism of action could have important implications for behavioral therapies. For example, one behavioral strategy used to counter impulsivity is reward bundling, which is typically used in instances where an individual has several consecutive choices to make between a small, immediate and large, later reward (e.g. the subjective pleasure of having a drink vs. the pleasure of feeling healthy and not having a hangover tomorrow). With reward bundling, the individual is told to treat each choice as if it were between the small, immediate reward (e.g. a drink) and the "bundled" value of *several* delayed rewards (e.g. feeling healthy and no hangovers for an *entire month*; see Monterosso & Ainslie, 2007, for a full explanation). Using such a strategy, the value of the delayed reward is increased, and the individual is more likely to choose the delayed option. However, if ethanol is not affecting delay processing, but rather impairs magnitude discrimination or greatly accelerates the rate of diminishing marginal utility, the increase in value for the "bundled" delayed rewards may be either unnoticed or markedly diminished. If so, a different behavioral strategy may be more effective.

Delay Discounting and the Brain.

Another reason to investigate the exact nature of ethanol's effects on delay discounting is that it could shed some light on the brain mechanisms involved. In both humans and animals, primarily rodents, several different brain regions have been

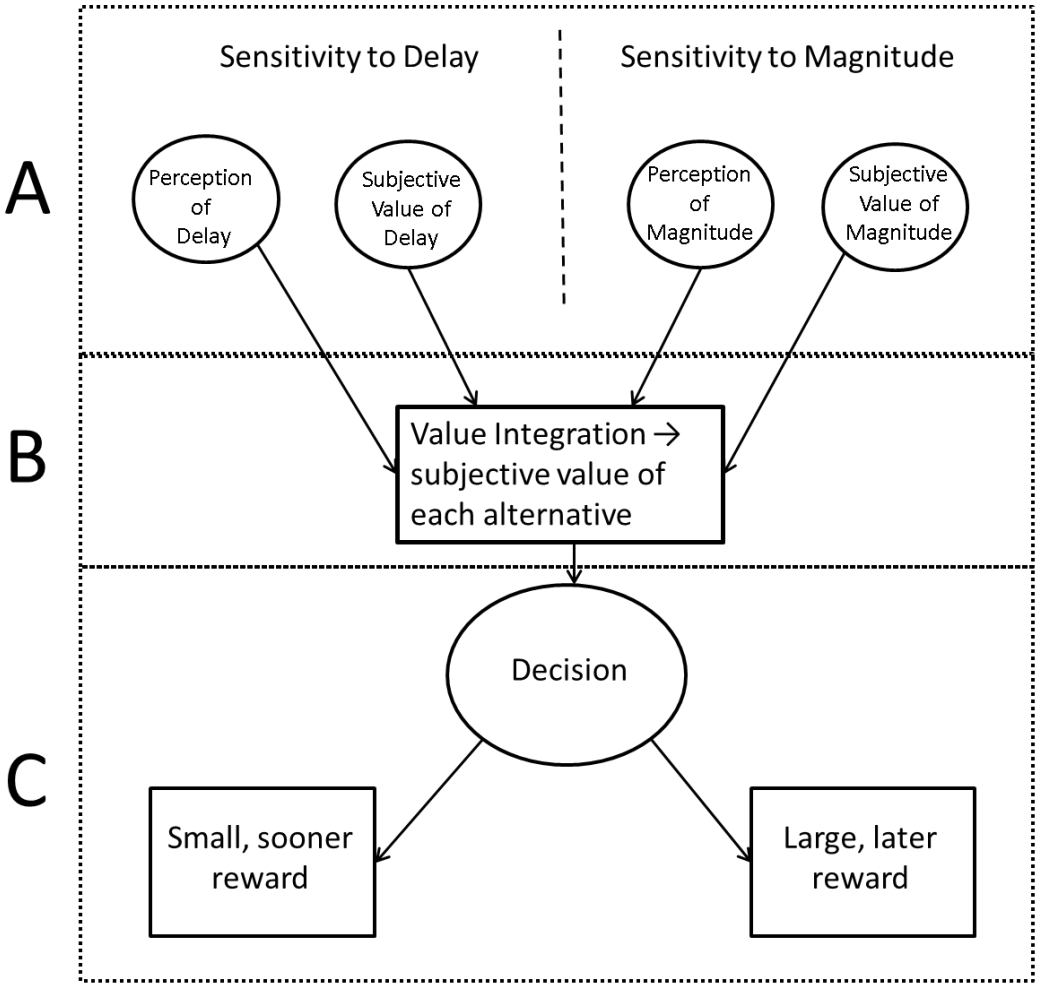


Figure 2. Simplified diagram indicating some of the theoretical substrates of delay discounting. **A)** The value of a reward is perceived through the lens of both sensitivity to delay and magnitude. Sensitivity to delay takes into account the ability of the individual to accurately perceive the delay and the subjective value the individual places on the delay. Similarly, sensitivity to magnitude takes into account the ability of the individual to accurately perceive the magnitude and the subjective value the individual places on the magnitude. **B)** The values of the magnitude and delay are integrated into a single value and compared to integrated value of the other reward. **C)** A decision is made based on the comparison of the two values.

implicated as important substrates for delay discounting. In human research, this comprises large swaths of the prefrontal cortex, including the medial, orbital, and dorsolateral prefrontal cortices (Figner et al., 2010; McClure et al., 2004). Areas of the insular and posterior parietal cortex have been implicated as well (McClure et al., 2004; Tanaka et al., 2004). Lastly, there is strong evidence that the ventral striatum plays a role in delay discounting (Kable & Glimcher, 2007; see Carter et al., 2010 for a review on human imaging studies and discounting).

Animal research has shown some overlap with the regions implicated in human studies. As with humans, the ventral striatum has been shown to be particularly important for delay discounting, and this is especially true of the ventral striatal subregion known as the nucleus accumbens core (Basar et al., 2010). However, other regions of the basal ganglia, such as the dorsomedial striatum and the subthalamic nucleus, are also thought to play a role (Eagle & Baunez, 2010; Winstanley et al., 2005). Additionally, several regions of the limbic system, including the basolateral amygdala and the hippocampus, have been implicated (Cheung & Cardinal, 2005; Winstanley et al., 2004). Lastly, despite the notable differences between human and rodent prefrontal cortex (Uylings et al., 2003), evidence from rodent literature suggests that regions of both the orbitofrontal and medial prefrontal cortices are likely involved (Churchwell et al., 2009; Mobini et al., 2002; see Cardinal, 2006; Eagle & Baunez, 2010, for reviews of this literature).

Within the animal literature, a few studies have sought to isolate the individual contributions of delay and magnitude processing to delay discounting, often using mathematics to model the contributions of the individual processes (see Ho et al., 1999;

Killeen, 2009; Logue, 1984; and Appendix 1 for descriptions of some of these models). These studies primarily focused on the ventral striatum, specifically, the nucleus accumbens core (AcbC), and the orbitofrontal cortex (OFC), and provided the impetus to examine these regions in this dissertation. Accordingly, a more thorough discussion of these two brain regions is warranted, from the perspective of both human and animal research. Specifically, it is important to know both the relationship of these two brain regions to delay discounting, as well as the role these two regions play in mediating the effects of acute ethanol administration.

Ventral Striatum. The ventral striatum is arguably the most heavily implicated region of the brain in delay discounting. Several studies using functional magnetic resonance imagining have shown the ventral striatum to be involved in delay discounting in humans (Jimura et al., 2013; Kable & Glimcher, 2007; Liu et al., 2012; McClure et al., 2004, 2007; Peters & Büchel, 2009; Prévost et al., 2010; Sripada et al., 2011; Tanaka et al., 2007; Wittmann et al., 2010). Of these studies, the majority have been satisfied to identify regions implicated in delay discounting but a subset have engaged in some theoretical discussion as to the precise role that the ventral striatum plays in discounting. Early studies suggested that ventral striatal activity[1] represented the value of an immediate option, and that ventral striatal activity was associated with impulsive choice (McClure et al., 2004, 2007). These authors argued that the ventral striatum was part of a network associated with immediate reward (the "β network") which was opposed by a network that calculated the relative value of rewards without any particular bias towards

[1] In functional magnetic resonance imaging, a signal known as the blood oxygen level dependent (BOLD) signal is taken to be a measure of neuronal activity. Although this is an indirect measure of neuronal activity, it has been shown to be well correlated with actual neuronal activity in macaques (Disbrow et al., 2000; Logothetis et al., 2001). Thus, although the two are not identical, I will use the term "activity" throughout this introduction when discussing the BOLD signal.

immediate rewards (the "δ network"). The existence of these two networks supported a previously proposed mathematical model of discounting known as the βδ model (Laibson, 1997).

However, subsequent research has suggested a different role for the ventral striatum. Kable & Glimcher (2007) found that ventral striatal activity was associated with the subjective value of the reward, regardless of whether or not that reward was delayed. The authors classified this subjective value as an integrative value that took into account both the delay and magnitude of the reward (Fig. 2b). Follow up studies suggested that the ventral striatum calculated subjective value for both delayed and probabilistic choices, but not for choices involving varying degrees of effort (Peters & Büchel, 2009; Prévost et al., 2010). Furthermore, one study explicitly showed that immediacy was not necessary to elicit ventral striatal activity in delay discounting (Sripada et al., 2011). Further research has suggested that the ventral striatum may be a reporter of the *relative* difference between two choices, although the literature has not made a distinction between delay or magnitude as the driving force in this process. For example, a study by Luo et al. (2009) found no ventral striatal activity when subjects compared immediate choices to preference matched delayed choices. Subjects also did not show ventral striatal activity when making difficult decisions between two choices that had similar subjective value (Marco-Pallarés et al., 2010). Lastly, while the ventral striatum does appear to be involved in valuation, it does not appear to be invoked during the actual decision-making process (Liu et al., 2012; see Fig. 2c). Thus, the current evidence from the human literature suggests that the ventral striatum reports the relative subjective value

difference between two temporal distinct choices, but beyond that is not involved in the downstream decision.

Imaging research in humans has three major difficulties that can be overcome through animal research: specificity, causality, and neuronal involvement. With animal models, one can target precise subregions of the ventral striatum and manipulate them to determine the causal necessity of that region. Lesion studies have suggested that the AcbC region of the ventral striatum is particularly important in delay discounting. Most of these animal studies have found that lesioning the AcbC increases discounting (Bezzina et al., 2007; Cardinal et al., 2001; da Costa Araújo et al., 2009; Galtress & Kirkpatrick, 2010; Pothuizen et al., 2005; but see Acheson et al., 2006). As with the human literature, the precise nature of this effect is a matter of debate. A study by Bezzina et al. (2007) suggested that the AcbC is important for sensitivity to the delay of the reinforcer, but is not involved in the sensitivity to the magnitude of the reinforcer. However, a study by Galtress & Kirkpatrick (2010) found the opposite. Unfortunately, both studies used tasks that required sensitivity to both delay *and* magnitude, making it difficult to dissociate the two. To overcome this limitation, a study by da Costa Araújo et al. (2010) used two separate tasks to independently assess sensitivity to delay and sensitivity to magnitude. One of the tasks was originally developed by Mazur (1984) to assess sensitivity to delay, and the second task was similarly structured but designed to measure sensitivity to magnitude. The authors did not directly manipulate any brain regions, but did measure c-fos counts as a measure of neuronal activity in the AcbC after exposure to the two separate tasks. Their results supported the earlier study by Bezzina et al. (2007) that showed that the AcbC was important for sensitivity to delay, but not

magnitude. However, other research has shown that neuronal firing in the ventral striatum tracks the subjective value of the chosen option regardless of whether it is a choice between two reinforcers of different magnitudes, or two reinforcers with different delays (Roesch et al., 2009).

Orbitofrontal cortex. Another region of the brain that has been intimately associated with delay discounting is the OFC. However, this region is difficult to define. While the ventral striatum is easily located in most mammals, the OFC does not have as many landmarks to distinguish it from other regions of the prefrontal cortex. This makes it difficult to interpret the human literature, which alternately calls the region orbitofrontal, ventromedial prefrontal, and even simply medial prefrontal cortex. Unfortunately, these three terms often include overlapping but partially distinct areas of the cortex (Kringelbach, 2005). Thus, it can be difficult to precisely locate where a change in activity occurred, or even whether that activity occurred in the OFC at all. That being said, several human imaging studies have explicitly implicated the OFC cortex in delay discounting (McClure et al., 2004, 2007; Peters & Büchel, 2009; Sripada et al., 2011; Wilbertz et al., 2012), and several more human imaging studies have found some association with a more broadly defined 'analogue' of the OFC (e.g. ventromedial prefrontal cortex; Jimura et al., 2013; Liu et al., 2012; Luhmann et al., 2008; Prévost et al., 2010; Wittmann et al., 2010). The OFC's role in discounting appears to be roughly similar to that of the ventral striatum, although there are a number of differences. As with the ventral striatum, McClure and colleagues (2004) suggested that the medial OFC was involved in choices between alternatives that included an immediate alternative (the β network). However, the lateral OFC appeared to be more associated with choices

between alternatives where both were delayed to some degree (the δ network). Kable & Glimcher (2007) found very little role for the OFC; only the most dorsal aspects seemed involved in subjective valuation. Nonetheless, three follow up studies did find a role for the OFC in subjective valuation (Peters & Büchel, 2009; Prévost et al., 2010; Sripada et al., 2011), although one notable difference from the ventral striatum was that Sripada et al. (2011) found the OFC to be involved in both subjective valuation *and* the immediacy of the reward. In addition to imaging studies, two studies have examined delay discounting in individuals with natural brain damage to this region. One study, by Fellows & Farah (2005), found no differences between experimental and control subjects. However, this study looked at damage to the ventromedial prefrontal cortex, which did not include the entire OFC. A subsequent study by Sellitto et al. (2010) explicitly used subjects with OFC lesions, and found that they had a strong increase in delay discounting when compared to controls.

The animal literature on the OFC and delay discounting is mixed. Lesions of the OFC increase delay discounting (Kheramin et al., 2002; Kheramin et al., 2003; Mobini et al., 2002; Rudebeck et al., 2006), decrease delay discounting (Winstanley et al., 2004), and have no effect (Abela et al., 2013; Finger et al., 2011; Jo et al., 2013; Mariano et al., 2009). Some attempts have been made to reconcile these discordant data. A study by Mar et al. (2011) found that lesions of the lateral OFC increased discounting, while lesions of the medial OFC decreased discounting, suggesting that the subregion of the OFC that is lesioned is an important consideration. Indeed, recent studies by St. Onge & Floresco (2010) and Stopper et al. (2012) have shown that this distinction exists for probability discounting as well, which requires the medial OFC, but not the lateral OFC.

Additionally, a study by Zeeb et al. (2010) determined that the nature of a lateral OFC inactivation's effect on discounting depended on both the basal level of discounting and whether or not the delay to reinforcement was signaled by a cue that was present for the duration of the delay.

In addition to attempts to clear up the mixed literature, researchers have also examined the role the OFC plays in the various processes underlying delay discounting. Similar to the AcbC, researchers have investigated the role that the OFC plays in sensitivity to delay and magnitude. Like the AcbC, the research has suggested that the OFC plays a role in sensitivity to delay (Kheramin et al., 2002). However, unlike the AcbC, it also suggested that the OFC is important for sensitivity to magnitude as well. The same group further investigated this in two tasks that separately measured sensitivity to delay and magnitude, finding that both tasks increased c-fos counts in the OFC (da Costa Araújo et al., 2010).

In total, a large body of evidence has implicated these two regions in delay discounting. Within the human literature, the majority of evidence has suggested that both regions are involved with determining and/or reporting the subjective value of the reinforcer as a whole (Fig. 2b). However, these studies are for the most part silent regarding the relative contributions of delay and magnitude processing, although one study has suggested that the ventral striatum is involved in magnitude processing, but has no role in delay processing (Ballard & Knutson, 2009). On the other hand, animal research has explicitly pursued the role of these two brain regions in delay and magnitude processing (Fig. 2a). Although the evidence is mixed, it suggests that the AcbC is important for delay processing, while the OFC is important for both delay and magnitude

processing. Nonetheless, no study has demonstrated direct evidence that the AcbC and the OFC are causally linked to delay or magnitude processing.

A brief review of ethanol's interactions with the ventral striatum and orbitofrontal cortex.

These two brain regions are implicated in the processes underlying delay discounting, but it is also important to understand their role in the effects of acute ethanol administration. Ethanol affects the functioning of several brain regions and neurotransmitter systems, both directly and indirectly (Watson & Little, 2002). This includes the Acb as well as, to a lesser extent, the OFC. I will briefly summarize some of this literature here.

Ventral Striatum. Systemic ethanol administration has long been known to alter neuronal functioning of the Acb. Ethanol increases neuronal activity (humans: Gilman et al., 2008; rats: Ryabinin et al., 1997; but see Ryabinin et al., 2000) as well as release of dopamine (humans: Boileau et al., 2003; rats: Chiara & Imperato, 1986), serotonin (rats: Yoshimoto et al., 1992), and opioids in the ventral striatum (humans: Mitchell et al., 2012; rats: Méndez et al., 2010). Additionally, intra-Acb administration of various dopamine receptor agonists and antagonists alters self-administration of ethanol (Hodge et al., 1997; Levy et al., 1991), thus suggesting that this region has a direct role in some ethanol-induced behaviors. Furthermore, dopamine receptor antagonists alter delay discounting in their own right (van Gaalen et al., 2006; Wade et al., 2000), which, combined with the previous findings, suggests the possibility that ethanol's effects on discounting are mediated by Acb dopamine.

Orbitofrontal cortex. More work has focused on the role of the OFC in chronic ethanol use than in its acute effects (Volkow & Fowler, 2000). Nonetheless, ethanol

increases measures of neuronal activity in the OFC in rats (Ryabinin et al., 1997) and increases opioid levels in the OFC in humans (Mitchell et al., 2012). Furthermore, the authors of the latter study have suggested that, given that opioids increase delay discounting (Kieres et al., 2004), ethanol-induced increases in opioids in the OFC may be driving ethanol's effects on discounting.

Thus, acute ethanol can alter the physiology and function of the Acb and the OFC. Coupled with their role in the processes underlying delay discounting, these two brain regions make ideal choices to investigate the role of delay and magnitude sensitivity in delay discounting.

Research Questions.

Based on the aforementioned findings, I had two primary research questions. First, I wanted to know the manner by which ethanol increases delay discounting in rats. As mentioned above, it is unclear whether ethanol alters sensitivity to delay, sensitivity to magnitude, or both. To determine this, I used variants of the tasks developed by Mazur (1984) and da Costa Araújo et al. (2010) to explicitly investigate whether ethanol altered sensitivity to delay, sensitivity to magnitude, or both.

Second, I wanted to know the direct role that the AcbC and OFC play in sensitivity to delay and sensitivity to magnitude. These two regions have been heavily implicated in delay discounting and have also been linked to sensitivity to delay and sensitivity to magnitude, albeit indirectly. Furthermore, given that ethanol can alter neuronal activity and neurotransmitter release in these two regions, they are also prime candidates to mediate the effect of ethanol in these two processes. Thus, using the tasks

mentioned above, I explicitly investigated whether inactivation[2] of either of these two regions altered sensitivity to delay, sensitivity to magnitude, or both.

[2] I could have used several different manipulations instead of inactivation, including dopamine agonist/antagonist microinjections, opioid agonist/antagonist microinjections, or simply lesion of the regions of interest. I chose inactivation because a) like ethanol, but unlike lesions, the effect of inactivation on the brain region is mostly temporary, and b) I wanted to determine the effect of blocking the neuronal activity of the regions first before pursuing more fine-tuned manipulations involving dopamine or opioids.

Chapter 2. Effect of ethanol on sensitivity to delay and magnitude.

This chapter is based on the following publication:

Moschak TM, Mitchell SH (2013) Sensitivity to reinforcer delay predicts ethanol's suppressant effects, but itself is unaffected by ethanol. Drug Alcohol Depend 132: 22-28.

Contributions:

Suzanne Mitchell aided in study design and manuscript preparation and Alex Murray helped run the animals in the behavioral tasks.

Introduction.

As mentioned in the previous chapter, acute ethanol administration can increase impulsivity. Although studies using human subjects have not yielded clear results (Ortner et al., 2003; Richards et al., 1999; Dougherty et al., 2008; Reynolds et al., 2006b), the majority of published studies using animal subjects have indicated that acute ethanol increases impulsivity in a delay discounting model (Evenden and Ryan, 1999; Hellemans et al., 2005; Olmstead et al., 2006; Poulos et al., 1998; Tomie et al., 1998; but see Wilhelm and Mitchell, 2012); that is, ethanol increases choice of a small, immediate reward over a larger, delayed reward.

Although ethanol's ability to increase discounting is well reported in animals, mathematical models of delay discounting do not indicate whether increases in delay discounting occur through increases in sensitivity to delay or decreases in sensitivity to magnitude (Ho et al., 1999; Killeen, 2009; Logue et al., 1984), nor do these models

address the effects of ethanol. Further, the typical procedure cannot easily dissociate ethanol's effects on sensitivity to reinforcer magnitude and reinforcer delay, because both reward magnitude and delay length differ between choice alternatives. Consequently, existing theory and data are inconclusive as to whether changes in one or both types of sensitivity are responsible for increases in delay discounting. However, two studies report that ethanol increased choice of the smaller reinforcer when there was no delay for both the small and the large reward, implying decreases in sensitivity to magnitude (Evenden and Ryan, 1999; Tomie et al., 1998; see Fig. 1c). Unfortunately, in most studies examining ethanol's effects on delay discounting, equivalent delay conditions were not assessed.

Determining the effect of ethanol on these two components of delay discounting is important because it could both impact treatment strategy and help identify the brain regions by which ethanol has its effect on discounting. For example, a behavioral treatment focused on changing sensitivity to delay (e.g., Ainslie, 1975; Monterosso and Ainslie 1999, 2007) would be of little help if ethanol only acts on delay discounting by altering sensitivity to reinforcer magnitude. Furthermore, since different brain regions have been implicated in delay and magnitude sensitivity (see Cardinal et al., 2006 for a review), determining whether ethanol alters sensitivity to delay or magnitude could narrow down which brain regions to target using selective drugs or other procedures. Accordingly, ethanol's effects on these parameters were tested by examining the effects of systemic ethanol on two tasks that manipulate delay or magnitude independently. I predicted that ethanol would increase sensitivity to delay, decrease sensitivity to magnitude, or do both.

Some research has indicated that drug-naïve performance on a typical delay discounting task predicts ethanol's effects on behavior. For example, steep delay discounting predicts increased sensitization to the locomotor activating effects of ethanol in outbred mice (Mitchell et al., 2006). This is relevant because the motor response to ethanol can itself be predictive of subsequent ethanol consumption. For example, in humans, low response to ethanol's effects predicts future alcoholism (Schuckit, 1994). Similar findings have been found in rats: High alcohol drinking and preferring rats are less sensitive to the behaviorally suppressant effects of ethanol than low alcohol drinking and preferring rats, although they are more sensitive to the stimulant effects of low doses of ethanol (Rodd et al., 2004; Waller et al., 1986; for review see Crabbe et al., 2010). Based upon this literature, I hypothesized that either high sensitivity to delay or low sensitivity to magnitude (operationally, low adjusting delay or low adjusting magnitude) would predict resistance to ethanol's behaviorally suppressant effects (operationally, number of trials completed).

Methods.

Subjects

Male Long-Evans rats (n = 20) were purchased from Charles River Laboratories (Hollister, CA). Long-Evans rats were used because the majority of studies finding an effect of ethanol on delay discounting in rats used this strain (Hellemans et al., 2005; Olmstead et al., 2006; Tomie et al., 1998). Rats were 8 - 9 weeks old and weighed approximately 275-300 g upon arrival. Animals were housed two per cage in a temperature-controlled vivarium at Oregon Health & Science University. A 12:12 light-dark cycle was used (lights on at 0600 h). All procedures were approved by the

Institutional Animal Care and Use Committee and adhered to NIH Guidelines. Animals were acclimated to handling for one week and were subsequently food restricted to approximately 85% of their free-feeding weights for the duration of the study.

Apparatus

Eight operant chambers (25 x 32 x 26) housed in sound-attenuating cabinets (4 x 64 x 42 cm; Med Associates Inc., St. Albans, VT) were used. Each cabinet contained a fan for ventilation and white noise. The top, back and front panels of the operant chambers were made of clear acrylic; the front panel also served as the door to the chamber. The left and right panels were made of stainless steel. The left panel contained a central houselight (top), as well as left and right cue lights (top), levers (middle), and liquid receptacles (bottom). Between the receptacles there was a center nosepoke with a built-in cue light. The right panel contained a tone generator (not used) and a response clicker. The chamber had a grid floor with a metallic pan underneath filled with bedding. Two computer-controlled pumps filled with 20% w/v sucrose were connected to the liquid receptacles. Input/output was controlled using a program written in MED-PC (Med Associates Inc.).

Drugs

Ethanol (200 proof) was diluted to 20% v/v in 0.9% saline, and was administered intraperitoneally (i.p.) immediately before the test session (0.0, 0.6, 0.9 g/kg). Each dose was administered 3 times, order counterbalanced. Test sessions occurred on Tuesdays and Fridays, and non-injection sessions occurred on Mondays, Wednesdays, and Thursdays.

Procedure

Training. All animals were trained in three consecutive phases, and graduated from each phase after obtaining at least 45 reinforcers over two consecutive sessions. Animals first trained with independent concurrent VT-120 s FR-1 schedules operating for the left and right levers/feeders. Subsequently, the VT-120 s contingency was dropped and animals were required to nosepoke into a center hole before the FR-1 contingency would operate for the left or right levers. Animals then entered a final training phase on which the FR-1 contingency was alternated between left and right levers on each trial. Total training took 16.0 ± 1.1 sessions to complete.

Adjusting Tasks (Fig 3). After completing training, animals entered the experimental phase and were randomly assigned to either the adjusting delay (n = 9) or adjusting magnitude task (n = 9). Two animals were not assigned: one died for reasons unrelated to the experiment and the other was unable to complete training. The two tasks used were based on those used by Mazur (1984) and da Costa Araújo et al. (2010) and required animals to respond on discrete trials. In these two tasks, increased sensitivity to delay is measured as a decrease in adjusting delay, while decreased sensitivity to magnitude is measured as a decrease in adjusting magnitude (see Appendix 1).

Events in each trial were the same for the adjusting delay and adjusting magnitude tasks. Every trial began with the illumination of the center nosepoke. If the rat poked its nose into the center nosepoke, the light was extinguished and the lights above the left and right levers were illuminated (free choice trial). For the trial to continue, animals had to press one of the levers. After two consecutive choices of the same lever on free choice trials, a forced choice was introduced such that only the light over the nonselected lever was illuminated. Following a press on an illuminated lever, the lever light was

extinguished, the reinforcer was delivered according to the contingencies associated with the specific lever and task, and the intertrial interval (ITI) began. Responses on the non-illuminated lever were recorded but had no effect. The duration of the ITI was adjusted on a trial by trial basis to ensure that the period between lever choice and the start of the next trial always lasted 27 seconds. Animals performed the task until they completed 90 trials or until 90 minutes elapsed, whichever occurred first. The adjusting and variable levers were switched daily according to a pseudorandom schedule, with no lever being on the same side for more than two consecutive days.

Adjusting delay task (Figure 3A). A press on the variable lever resulted in a liquid sucrose reinforcer (75 µl) delivered 18 s or 2 s after the lever press (probability = .5). A press on the adjusting lever resulted in the same size liquid sucrose reinforcer delivered *t* s after the lever press, where *t* was equal to the adjusting delay time. The step size by which the adjusting delay altered across trials was calculated based on each animal's choices. Choice of the variable lever decreased the adjusting delay by 10%, while choice of the adjusting lever increased the adjusting delay by 10%. Percentages, rather than fixed values, were used to address the psychophysical relationship between delay length and perceived delay length. The adjusting delay was set to 10 s at the start of each session, and could not go below 0.75 s or above 22 s.

Adjusting magnitude task (Figure 3B). A press on the variable lever resulted in immediate delivery of a liquid sucrose reinforcer with a magnitude of 15 µl or 135 µl (probability = .5). A press on the adjusting lever resulted in immediate delivery of a liquid sucrose reinforcer with a magnitude of *m* µl, where *m* was equal to the adjusting magnitude. The adjusting magnitude step size was calculated based on each animal's

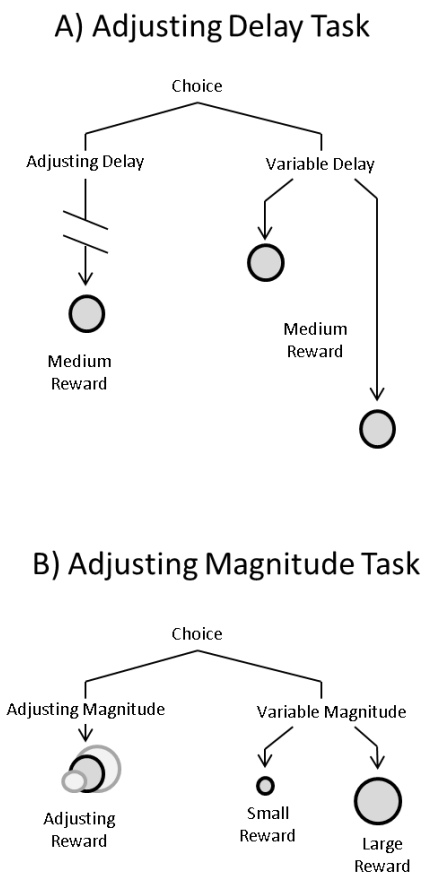


Figure 3. Diagram of the adjusting delay and adjusting magnitude tasks. **A)** The adjusting delay task. Animals chose between a medium reward after an adjusting delay, or a medium reward after either 2 or 18 s delay (p = 0.5 for either). **B)** The adjusting magnitude task. Animals chose between an immediate, adjusting reward, or an immediate small or large reward (p = 0.5 for either).

choices. Choice of the variable lever increased the adjusting magnitude by 10%, while choice of the adjusting lever decreased the adjusting magnitude by 10%. The adjusting magnitude was set to 75 µl at the start of each session, and could not go below 6 µl or above 165 µl.

Before testing began, individual animals had to have at least 5 days of stable data (determined by visual inspection). Animals required 35.7 ± 3.0 days to attain stability in the task.

Contingency Manipulations. Before ethanol administration, the sensitivity of the tasks to manipulation was verified by altering the values of the variable lever reward delays or magnitudes for animals in each task. Contingency manipulations were administered on Tuesdays and Fridays; on all other days animals were run on the normal task. In the adjusting delay task, animals were exposed to 2 s / 2 s, 2 s / 6 s, 6 s / 18 s, and 18 s / 18 s alternatives on the variable lever (order counterbalanced); for the adjusting magnitude task, animals were exposed to 15 µl / 15 µl, 15 µl / 45 µl, 45 µl / 135 µl, and 135 µl / 135 µl on the variable lever (order counterbalanced).

Data analysis

Contingency Manipulations. The contingency manipulations were analyzed with a 4 x 2 ANOVA (Manipulation condition x Session; the former variable compares each manipulation to the others, the latter variable compares each manipulation session to the preceding non-manipulation session).

Effect of Acute Ethanol. The primary dependent variable was the mean adjusting delay or magnitude value for the latter half of each session (last 45 trials). The latter half was used rather than the whole session in order to allow the animal time to achieve a

point of indifference between the two levers (although it should be noted that the results did not change if data for the entire session or first half were used). There were a few instances where animals did not complete all of the trials within the 90-min session. If fewer than 45 trials were completed, such sessions were dropped from the analysis (6 out of 162 injection sessions). If more than 45 were completed but animals did not complete the session, adjusting delay or magnitude values were calculated using data from trials after trial 45 (16 out of 162 injection sessions). Data were collapsed across session and analyzed using a 3 x 2 ANOVA (Dose x Injection; the "Injection" variable compared data from the injection days with data from the days immediately prior to the injection days to determine if there were any effects of injection itself). Similar analyses were performed using preference data (proportion choice of the variable lever). An additional series of 3 x 2 x 18 ANOVAs were performed to examine the effect of ethanol on the number of trials completed as a function of dose, injection and time during the session (number of trials completed was averaged into eighteen 5-minute bins). These were followed up with individual ANOVAs for each dose.

To determine the nature of the behaviorally suppressant effect of ethanol, a 3 x 2 x 10 ANOVA (Dose x Injection x Timebin) analysis was performed on the logarithmically transformed reaction times for each animal in each time bin. Reaction time to nosepoke and reaction time to lever press were also examined. Only the first 50 minutes of the task were used in this analysis because many animals completed the task shortly after that time, which introduced missing data into subsequent time bins.

Regression Analysis. A linear regression model using the adjusting delay or magnitude values averaged for the 10 days prior to any ethanol exposure was performed

to predict the number of trials completed as well as the reaction times after ethanol administration for each dose. Number of trials completed or reaction time on the pre-injection day was also used as a predictor variable in this model to control for differences in baseline responding in the task. Because animals recovered from ethanol's behaviorally suppressant effects over the course of the session, only the total number of trials completed or reaction times in the first 20 min of the session were used in the analysis. The use of 20 min was based on the duration of the distribution phase seen in rat brain tissue after i.p. ethanol (lasting roughly 20 min; Adalsteinsson et al., 2006, using 1.0 g/kg) as well as the rate of recovery seen in the data (see Fig. 6).

Results.

Contingency Manipulations. There were significant effects of the contingency manipulations for both the adjusting delay and adjusting magnitude tasks (Manipulation x Session: delay: $F(3,24) = 9.20$, $p < .001$; magnitude: $F(2.29,18.33) = 10.50$, $p = .001$). Bonferroni post hoc tests found significant effects for the 18 s / 18 s, 15 µl / 15 µl, and 135 µl / 135 µl conditions compared to the session prior to manipulation, suggesting that these tasks were able to measure acute changes in behavior (see Fig. 4).

Effect of Acute Ethanol. Animals reached approximate indifference by the last 45 trials in both tasks after injection (magnitude task: 53.2 ± 1.8% preference for variable lever, one-sample t-test vs. 50%: $t(8) = -1.75$, $p = .119$; delay task: 52.1 ± 1.5% preference for variable lever, $t(8) = -1.36$, $p = .210$). There were no effects of ethanol on adjusting delay or adjusting magnitude, calculated from these final 45 trials of the session, at any dose (Dose x Injection, adjusting delay: $F(2,16) = .84$, $p = .450$; Dose x Injection, adjusting magnitude: $F(2,16) = .06$, $p = .946$; see Fig. 5). Ethanol also did not

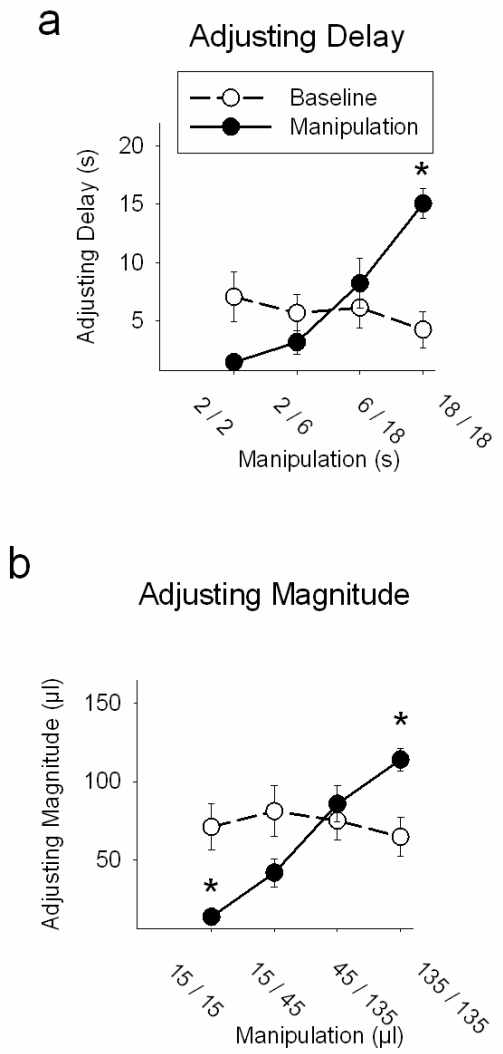


Figure 4. **a)** Effect of delay manipulations on adjusting delay. Baseline days used 2 / 18 s for the variable lever (dashed lines, open circles). The manipulation days used 2 / 2 s, 2 / 6 s, 6 / 18 s, and 18 / 18 s (solid lines, closed circles). Only the 18 / 18 s manipulation had a significant effect on adjusting delay, although the 2 / 2 s manipulation was significant before Bonferroni correction. **b)** Effect of magnitude manipulations on adjusting magnitude. Baseline days used 15 / 135 µl for the variable lever (dashed lines, open circles). The manipulation days used 15 / 15 µl, 15 / 45 µl, 45 / 135 µl, and 135 / 135 µl (solid lines, closed circles). Both the 15 / 15 µl and 135 / 135 µl manipulations had a significant effect on adjusting magnitude.

affect preference ratios for either task (Fs < 1.4, ps > .27). However, ethanol did dose-dependently decrease the rate at which trials were completed early in the session, followed by a compensatory increase later in the session as shown by the Dose x Injection x Time bin analyses (adjusting delay: $F(34,272) = 11.96$, $p = < .001$; adjusting magnitude: $F(34,272) = 4.62$, $p < .001$; see Fig. 6). Examining the saline, 0.6 and 0.9 g/kg dose conditions separately revealed that, although this effect was by far the strongest at 0.9 g/kg, all doses had an effect on the number of trials completed per time bin on the adjusting delay task (Saline, $F(17,136) = 1.98$, $p = .016$; 0.6 g/kg, $F(17,136) = 4.41$, $p < .001$; 0.9 g/kg, $F(17,136) = 26.04$, $p < .001$; both 0.6 and 0.9 g/kg were significantly different from saline: saline vs. 0.6 g/kg: $F(17,136) = 5.44$, $p < .001$; saline vs. 0.9 g/kg: $F(17,136) = 12.07$, $p < .001$). Only the effects of the 0.6 and 0.9 g/kg dose conditions were significant for the adjusting magnitude task (Saline, $F(17,136) = 1.21$, $p = .267$; 0.6 g/kg, $F(17,136) = 4.24$, $p < .001$; 0.9 g/kg, $F(17,136) = 10.55$, $p < .001$; both 0.6 and 0.9 g/kg were significantly different from saline: saline vs. 0.6 g/kg: $F(17,136) = 6.95$, $p < .001$; saline vs. 0.9 g/kg: $F(17,136) = 3.05$, $p < .001$). Bonferroni post hoc tests comparing pre-injection day to injection day only found significant differences in individual time bins at the 0.9 g/kg dose. In the adjusting delay task, trials completed were significantly different from pre-injection in the 5 – 30 min time bins and in the 65 - 75 min time bins. In the adjusting magnitude task, trials completed were significantly different from pre-injection in the 5, 15, and 85 min time bins. Additionally, ethanol dose-dependently increased time to initiate the trial early in the session in the adjusting magnitude task (Dose x Injection x Time bin, $F(18,144) = 2.40$, $p = .002$) and for the entire first 50 min of the adjusting delay task (Dose x Injection, $F(2,16) = 9.92$, $p = .002$).

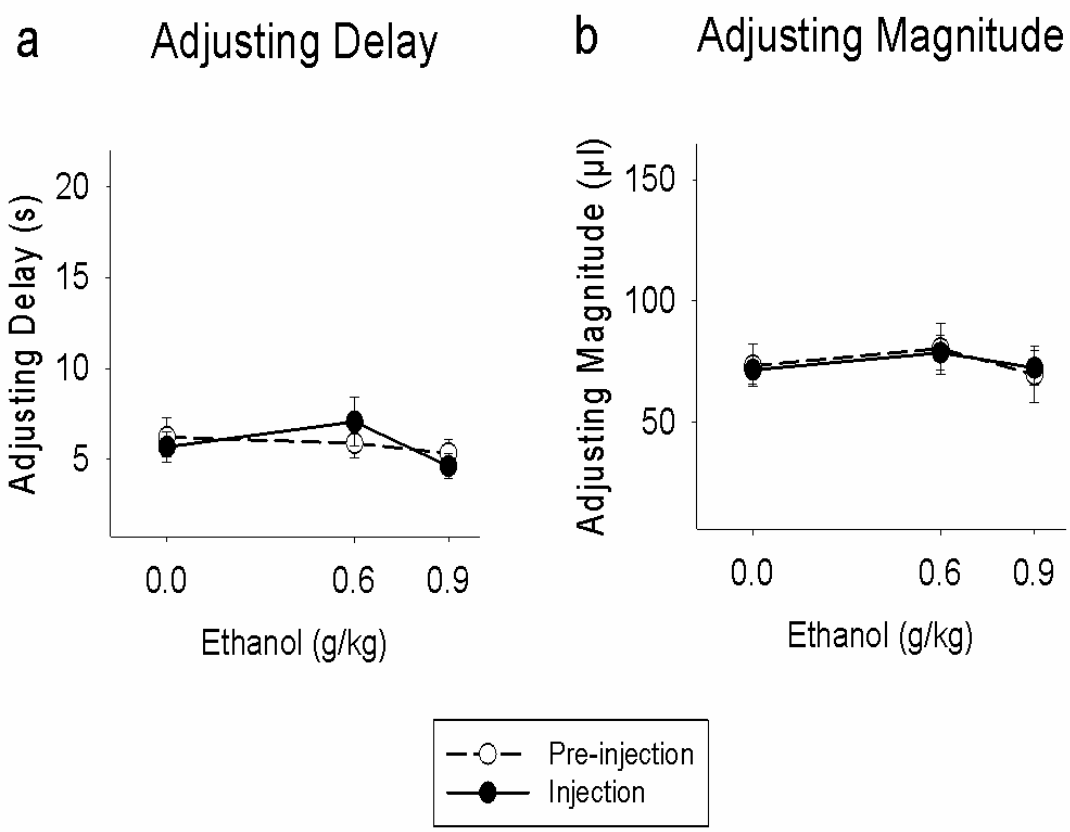


Figure 5. Ethanol did not affect adjusting delay **(a)** or adjusting magnitude **(b)**.

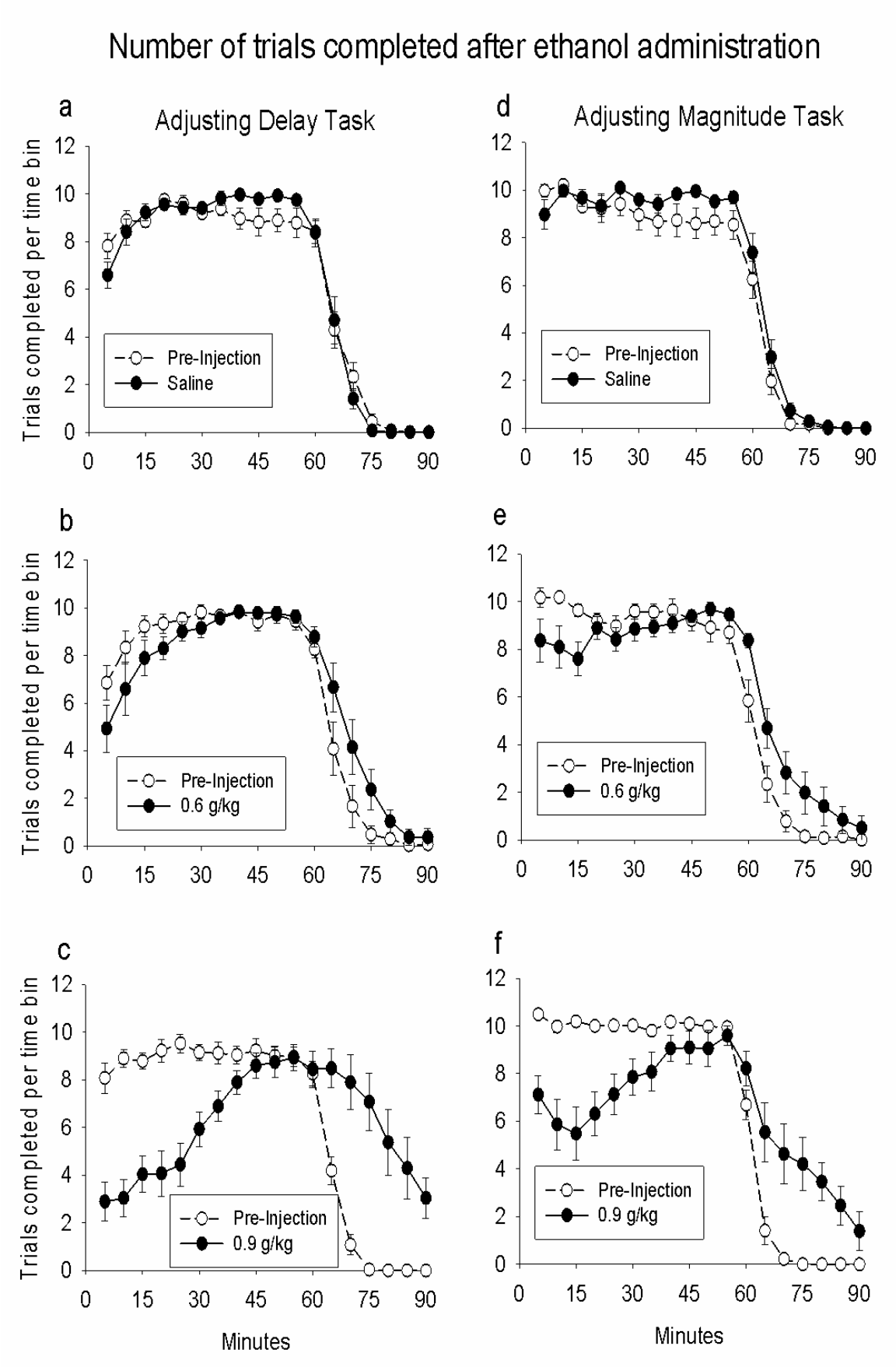

Number of trials completed after ethanol administration
a
Adjusting Delay Task
d
Adjusting Magnitude Task
b
e
c
f
Trials completed per time bin
Pre-Injection
Saline
0.6 g/kg
0.9 g/kg
Minutes
0
15
30
45
60
75
90
2
4
6
8
10
12

Figure 6. Ethanol dose-dependently decreased the number of trials completed per time bin (with a subsequent rebound later on) in both tasks. On the days before ethanol injection, animals typically finished the task within 75 min (dashed lines). Both doses of ethanol reduced the number of trials completed and delayed the amount of time it took animals to complete the task; this effect was particularly strong for the 0.9 g/kg dose (**b**, **e**; **c**, **f**).

Ethanol also dose-dependently increased lever choice reaction time for the entire first 50 min of the adjusting magnitude task (trend, Dose x Injection, $F(2,16) = 2.91$, $p = .084$) and early in the session in the adjusting delay task (Dose x Injection x Time bin, $F(18,144) = 1.92$, $p = .019$). These effects were primarily restricted to 0.9 g/kg ($Fs > 2.71$, $ps < .021$), although modest effects at other doses were seen (adjusting delay, trial initiation, saline: Time bin x Injection: $F(9,72) = 2.29$, $p = .025$; adjusting magnitude, trial initiation, 0.6 g/kg: Time bin X Injection: $F(9,72) = 2.79$, $p = .007$). This shows that 0.9 g/kg generally slowed both trial initiation and choice. Number of trials completed and the log reaction time were highly correlated after 0.9 g/kg ethanol ($r = -.80$, $p < .001$).

Regression Analysis. The average adjusting delay on the 10 days before ethanol administration was significantly predictive of the number of trials completed in the first 20 min of 0.9 g/kg ethanol sessions when the number of trials completed on pre-injection days were statistically controlled ($t = -2.62$, $p = .040$; see Fig. 7). No significant relationships were seen for any other dose, for any of the adjusting magnitude data, or for any of the reaction time data.

Discussion.

The current study found that ethanol had no effect on sensitivity to delay or sensitivity to magnitude. These findings are surprising given the previous literature showing that ethanol can affect delay discounting. However, although many published studies have reported an effect of ethanol on delay discounting, these effects are typically fairly small (e.g. Evenden and Ryan, 1999; Hellemans et al., 2005; Olmstead et al., 2006; Tomie et al., 1998) and at least one published study has failed to find any effect (Wilhelm and Mitchell, 2012). It may be that the procedural differences between the tasks used in this

study and those used in other studies are sufficient to obscure such modest effects. For example, in the tasks used in this study the value of the delay or magnitude changed as a function of the animals' choices, whereas in the aforementioned studies they did not (with the exception of Wilhelm and Mitchell, 2012, which also found no effect of ethanol). If such a procedural difference is sufficient to alter the effect of ethanol, then it may suggest that performance in the tasks used here relies on different neural substrates, or that ethanol's effects on delay discounting in other tasks are via a means other than delay or magnitude sensitivity. It should also be noted that attaining stability in these tasks took longer than some other studies (e.g. 35 days in the current study compared to 25 days in Evenden & Ryan, 1999 and 18 days in Tomie et al., 1998; however Poulos et al., 1998 took 40 days); thus overtraining may be a valid concern. However, further work would be needed to test these hypotheses.

While there were no effects of ethanol on delay or magnitude sensitivity, this study did find that rats with high sensitivity to delay were resistant to ethanol's behaviorally suppressant effects. A similar relationship was not seen in the adjusting magnitude task. The relative specificity of this effect may have implications for other studies showing delay discounting to be predictive of drug effects, since the current study suggests that it is sensitivity to delay, rather than sensitivity to magnitude, that is particularly important.

The fact that high sensitivity to delay predicts low response to ethanol's behaviorally suppressant effects may add to the larger literature on high and low responders to ethanol (for reviews of this literature, see Crabbe et al., 2010; Schuckit et al., 2004). It has previously been shown that rats selectively bred for high alcohol

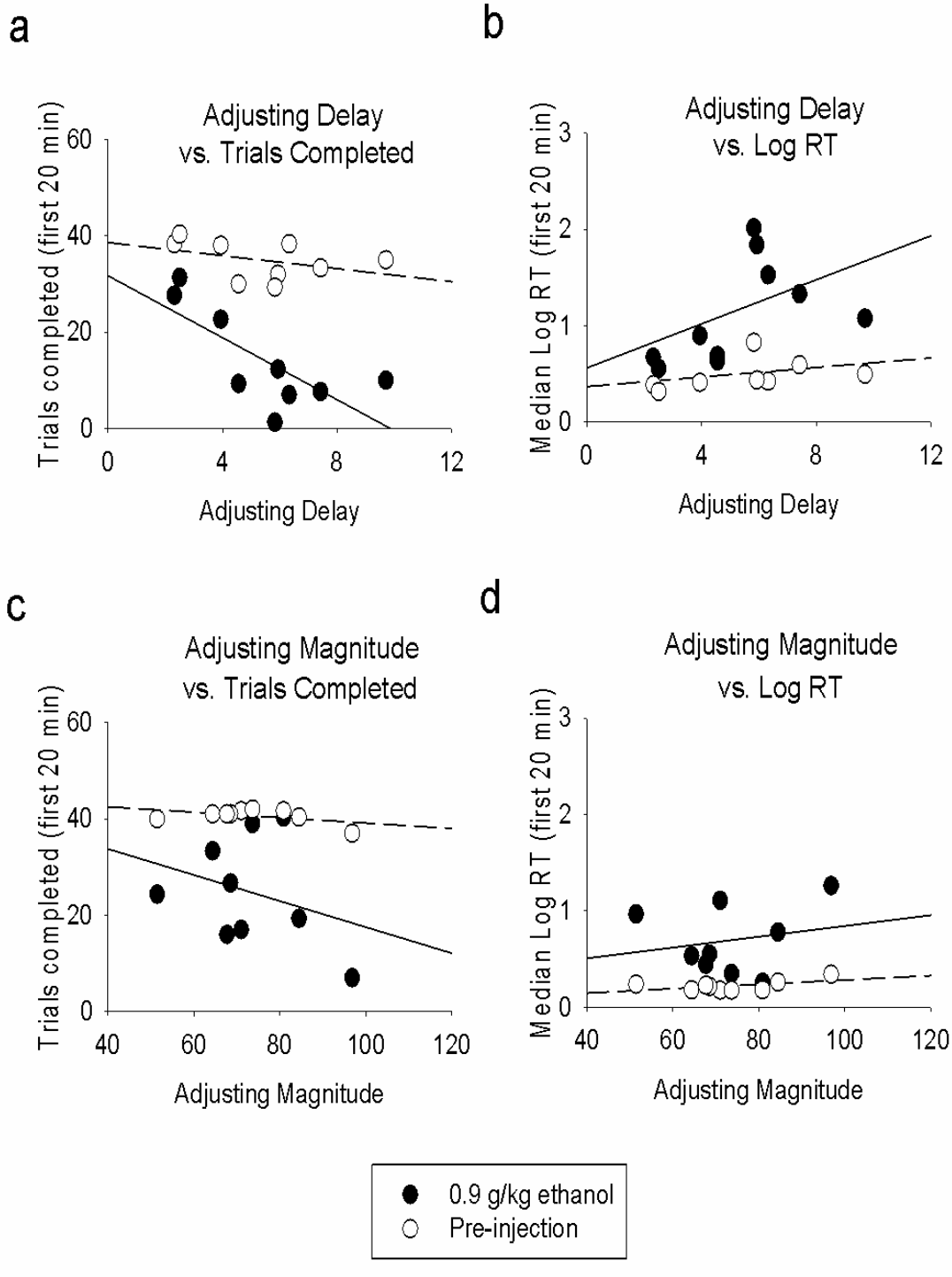

a
Adjusting Delay
vs. Trials Completed
Trials completed (first 20 min)
60
40
20
0
0
4
8
12
Adjusting Delay
b
Adjusting Delay
vs. Log RT
Median Log RT (first 20 min)
3
2
1
0
0
4
8
12
Adjusting Delay
c
Adjusting Magnitude
vs. Trials Completed
Trials completed (first 20 min)
60
40
20
0
40
60
80
100
120
Adjusting Magnitude
d
Adjusting Magnitude
vs. Log RT
Median Log RT (first 20 min)
3
2
1
0
40
60
80
100
120
Adjusting Magnitude
0.9 g/kg ethanol
Pre-injection

Figure 7. Relationship between average adjusting value and number of trials completed or reaction time in the first 20 min after 0.9 g/kg ethanol. Animals with high sensitivity to delay (low adjusting delay) were resistant to ethanol's effects on responding (**a**), but there was no relationship with reaction time (**b**). There was no relationship between sensitivity to magnitude and ethanol's behaviorally suppressant effects or effects on reaction time (**c**, **d**).

consumption exhibit higher ethanol-induced locomotor activity than their low-consuming counterparts (Agabio et al., 2001; Krimmer and Schecter, 1991; Quintanilla, 1999; Rodd et al., 2004; Waller et al., 1986). Furthermore, two of the aforementioned studies also examined doses high enough to result in activity suppression; in both cases the low-consuming lines were more suppressed by ethanol than the high-consuming lines (Rodd et al., 2004; Waller et al., 1986). Thus, there appears to be a genetic link between locomotor response to ethanol and consumption of ethanol, and the current study suggests that the locomotor response to ethanol may also be related to sensitivity to delay (although it should be noted that the Long-Evans strain used in this study only exhibits ethanol-induced decreases in locomotor activity, not biphasic increases and decreases; Duncan & Cook, 1981).

Complementing these findings are several other studies that have linked delay discounting to ethanol *consumption* (for reviews of this literature see Lejuez et al., 2010; Mitchell, 2011). In humans, individuals dependent upon alcohol show steeper delay discounting than controls (Bjork et al., 2004; Mitchell et al., 2005; Petry, 2001). In animals, steeper delay discounters subsequently consume more ethanol (Poulos et al., 1995; but see Diergaarde et al., 2012). Along similar lines, animals that have been bred for high alcohol consumption also show steeper delay discounting than their low alcohol consumption counterparts (Oberlin and Grahame, 2009; Wilhelm and Mitchell, 2008; but see Wilhelm et al., 2007; Wilhelm and Mitchell, 2012). Indeed, both replicates of the high alcohol drinking [HAD] rat lines have steeper discounting (Wilhelm and Mitchell, 2008), are less sensitive to the locomotor suppressing effects of ethanol (Rodd et al., 2004), and, of course, drink more ethanol than their low alcohol drinking [LAD]

counterparts (McKinzie et al., 1998). It may therefore prove fruitful for future studies to examine the direct contributions (and degree of overlap) of these different traits to ethanol consumption in both selectively bred and outbred animals.

This study has a number of limitations. First, while animals were able to appropriately react to most contingency manipulations, there was no significant effect of reducing the length of the variable adjusting delay (Fig. 4). Although I suspect this reflects a lack of power rather than a true lack of effect ($p = 0.027$ *before* Bonferroni correction), I acknowledge that animals may have had difficulty decreasing adjusting delay in response to manipulations. Second, the relationship between adjusting delay and trials completed did not reach significance between adjusting delay and reaction time. Nonetheless, I feel that number of trials completed captures more of ethanol's behaviorally suppressant effects than reaction time because five of the nine adjusting delay animals had sessions in which they did not respond for the entirety or majority of the 20 min period (and thus had no measurable reaction time). Therefore, while ethanol clearly reduced trials completed during this time, its effect on reaction time was unknown for a portion of the data. Third, the tasks incorporate probability to dissociate delay and magnitude. Thus, it is difficult to completely dissociate the effects seen from sensitivity to probability. However, it seems unlikely that the effects seen rely on sensitivity to probability alone, because increased preference for the probabilistically variable option in the adjusting delay task (lower adjusting delay) predicted resistance to ethanol's effects (Fig. 7a), while preference for the variable option in the adjusting magnitude task (higher adjusting magnitude) was not related (Fig 7b). Furthermore, a previous study using these tasks found that the nucleus accumbens core had higher c-fos counts in animals exposed

to the adjusting delay but not adjusting magnitude task, further suggesting that probability alone was not responsible for the differences seen (da Costa Araújo et al., 2010). Nonetheless, it is still possible that an interaction between delay and probability is at work here, and additional studies would be needed to fully disentangle this.

In summary, there were two primary findings. First, ethanol did not affect sensitivity to delay or magnitude in two independent tasks. I believe this suggests that the measures of delay and magnitude sensitivity used in this study are differentially affected by ethanol than other measures of delay discounting. Thus, it may be that ethanol only affects the synergistic confluence of the neural substrates involved in both sensitivity to delay and magnitude together (as seen in standard delay discounting tasks), or it may be that the independent measures of sensitivity to delay or magnitude require entirely different neural substrates. The second finding showed that animals more sensitive to delay are less sensitive to ethanol's behaviorally suppressant effects. This finding fits with and expands existing literature, and supports the idea that sensitivity to delay and sensitivity to ethanol's behaviorally suppressant effects may be driven (at least in part) by the same underlying process.

Chapter 3. Effect of nucleus accumbens core or lateral orbitofrontal cortex inactivation on delay discounting, sensitivity to delay, and sensitivity to magnitude

This chapter is based on the following manuscript submitted for publication:

Moschak T.M., Mitchell, S.H. (2014) Nucleus accumbens core inactivation decreases delay discounting in rats with low baseline levels of discounting, without affecting performance in independent assessments of sensitivity to delay or magnitude. Behavioral Brain Research, submitted.

Contributions:

Suzanne Mitchell aided in study design and manuscript preparation, Wesley Wenzel, Janelle Payano-Sosa, Katrina Bettencourt and Mary Ann Reeves helped run the animals in the behavioral tasks, Katrina Bettencourt, Mary Ann Reeves and Robbie Mills assisted with brain tissue processing, and Christie Pizzimenti, Mary Ann Reeves and Robbie Mills helped verify cannulae placement.

Introduction.

The previous chapter delved into the relationship between ethanol and sensitivity to delay and magnitude. This chapter focuses on the relationship between discrete brain regions and sensitivity to delay and magnitude. The two regions of interest are the nucleus accumbens core (AcbC) and the orbitofrontal cortex (OFC), based on their role in both substance abuse and delay discounting (Crews & Boettiger, 2009; Winstanley et al., 2006).

The AcbC is thought to play a role in promoting choice of the larger, later reward, based on evidence that *lesioning* the AcbC *decreases* choice of the larger, later reward (Bezzina et al., 2007; Bezzina et al., 2008; Cardinal et al., 2001; da Costa Araújo et al., 2009; Galtress & Kirkpatrick, 2010; Pothuizen et al., 2005; but see Acheson et al., 2006). Conversely, the role of the OFC is less clear. Some studies have found that lesions increase delay discounting (Kheramin et al., 2002; Kheramin et al., 2003; Mobini et al., 2002; Rudebeck et al., 2006), one has found that lesions decrease discounting (Winstanley et al., 2004), and some have found no effect (Finger et al., 2011; Abela et al., 2013; Jo et al., 2013; Mariano et al., 2009). These discrepant findings have been suggested to be the result of procedural differences (Zeeb et al., 2010) and/or the regional specificity of the lesions (Mar et al., 2011).

Although both the AcbC and OFC have been implicated in discounting, it is unknown whether their role in discounting is mediated through sensitivity to reinforcer delay or sensitivity to reinforcer magnitude. Both delay and magnitude sensitivity are important components of delay discounting (Ho et al., 1999; Killeen, 2009; Logue et al., 1984), and determining the neurological underpinnings of these components may increase the understanding of discounting and its role in various behavioral disorders. Mathematical analysis of delay discounting data has suggested that the AcbC may modulate sensitivity to delay, while the OFC may modulate sensitivity to delay *and* magnitude (Bezzina et al., 2007; Kheramin et al., 2002; but see Galtress & Kirkpatrick, 2010). However, because these studies used a delay discounting task that manipulated delay and magnitude simultaneously, they were unable to *directly* measure sensitivity to delay or magnitude in isolation. To address this issue, a recent study by da Costa Araújo

et al. (2010) developed a method to directly assess sensitivity to delay or magnitude using two separate tasks. In agreement with the previous studies, the authors found that rats exposed to the task requiring sensitivity to delay had increased neuronal activity (c-fos counts) in both the AcbC and OFC, but that rats exposed to the task requiring sensitivity to magnitude only had increased neuronal activity in the OFC. Nonetheless, because the authors did not directly manipulate either of the two brain regions, a direct causal role in sensitivity to delay and magnitude has not yet been established.

To determine the existence of such a role, these regions were inactivated separately in rats and their performance was measured in a task measuring sensitivity to delay *and* magnitude (delay discounting), and in two tasks that separately measured sensitivity to delay *or* sensitivity to magnitude. I hypothesized that AcbC inactivation would increase delay discounting and increase sensitivity to delay, but have no effect on sensitivity to magnitude. Additionally, I hypothesized that OFC inactivation would impact each of the three measures, but did not specify a direction for these effects due to the mixed nature of the OFC discounting literature.

I was also interested in the role that individual differences might play in these effects. Individual differences in delay discounting have been correlated with differences in medial prefrontal cortex physiology (Loos et al., 2010a; Simon et al., 2013), a region that receives projections from the OFC and sends projections to the OFC and AcbC (Öngür & Price, 2000). Furthermore, a recent study by Zeeb et al. (2010) found that inactivation of the lateral OFC (lOFC) decreased delay discounting in animals that discounted delayed rewards more at baseline, but had no effect on animals that exhibited low levels of discounting at baseline. Therefore, I investigated whether individual

differences in behavior would modulate the effects of inactivation on performance in any of the three tasks.

Methods.

Subjects.

Male Long Evans rats (n = 96) were purchased from Charles River Laboratories (Hollister, CA). All animals arrived in and were housed in the same conditions as described in Chapter 2. Animals were run in two separate cohorts (n = 48 each).

Apparatus

This study used the same operant chambers that were used in Chapter 2.

Procedure.

Training. Training was identical to that done in Chapter 2

General Procedure (Fig. 8). The procedure was based on that used in Chapter 2, with a few important differences. First, an additional delay discounting task was included as a control task that simultaneously altered delay *and* magnitude (based on the within sessions task developed by Evenden & Ryan, 1996). Second, variable delays for the adjusting delay tasks were switched from 2 and 18 s to 0 and 20 s. This was done because the immediacy of the reward (i.e. 0 s delay) is suggested to play an important role in the effects seen in delay discounting studies (Laibson, 1997; McClure et al, 2004; Monterosso & Ainslie, 2007) and because typical delay discounting procedures have an immediate option (including each of the studies referenced in the introduction). In order to mimic the change seen in adjusting delay, the adjusting magnitude task also changed from 15 and 135 µl to 0 and 150 µl. After completing training, animals were randomly

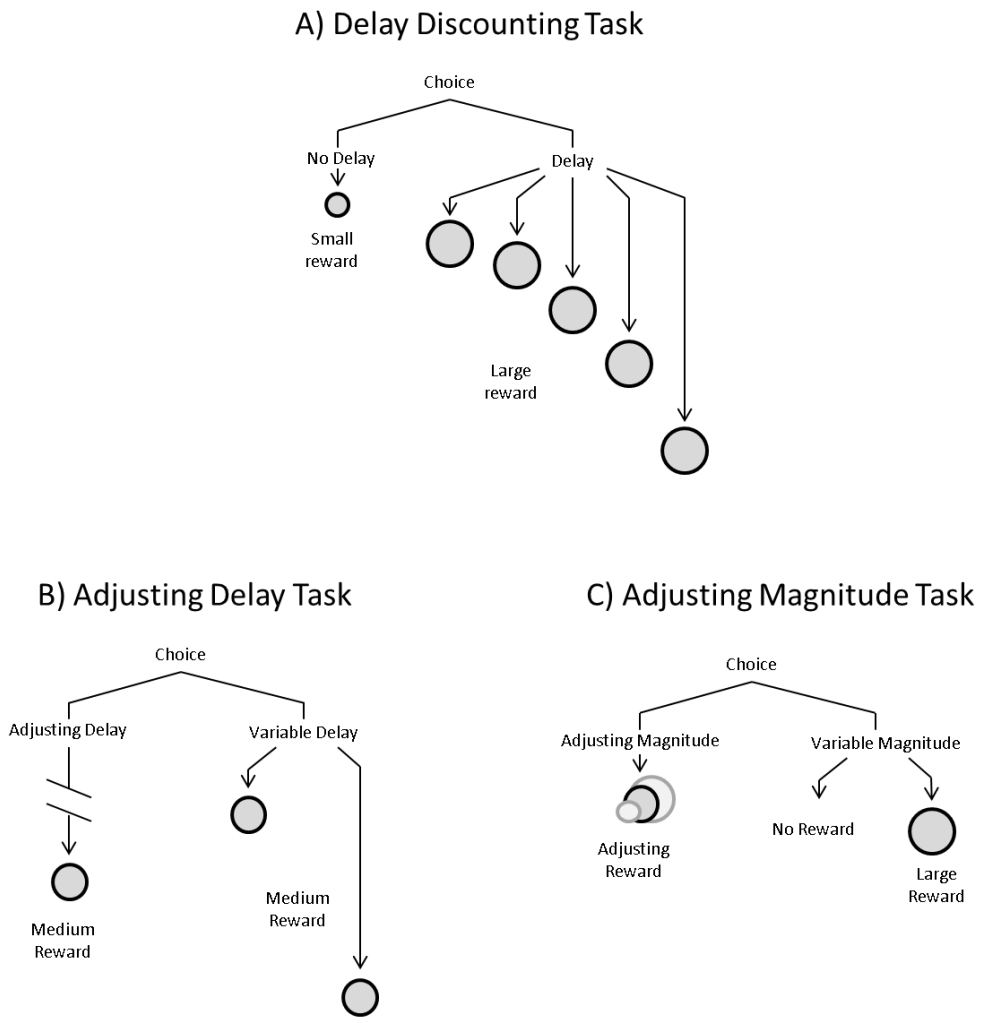


Figure 8. Task schematics. **A)** The within sessions delay discounting task. Animals chose between an immediate, small reward and a large, delayed reward. The length of the delay increased every 12 trials (0, 2.5, 5, 10, 20 s). **B)** The adjusting delay task. Animals chose between a medium reward after an adjusting delay, or a medium reward after either no delay or a 20 s delay (p = 0.5 for either). **C)** The adjusting magnitude task. Animals chose between an immediate, adjusting reward, or an immediate large reward / no reward (p = 0.5 for either).

assigned to one of these three tasks: within sessions task (discounting task), adjusting delay task (delay task), or adjusting magnitude task (magnitude task).

All tasks used the same basic structure. Each trial began with the illumination of the central aperture. If the rat poked its nose into the aperture, the light was extinguished and the lights above both levers were illuminated (free choice). If the animal pressed one of the levers, the lights were extinguished and the contingencies associated with that lever went into effect, followed by an intertrial interval (ITI). After two consecutive choices of the same lever, a forced trial occurred wherein only the light over the previously nonselected lever was illuminated and only responses on that lever resulted in a continuation of the trial. The ITI was adjusted to ensure that the duration from the lever press to the next trial was always 27 s. Animals performed in the task until 60 trials were completed or until 60 min had elapsed, whichever occurred first.

Within sessions task (Fig. 8a). All animals were assigned an immediate and a delayed reward lever. A press on the immediate reward lever resulted in immediate delivery of 50 µl sucrose. A press on the delayed reward lever resulted in delivery of 150 µl sucrose after a delay that increased after each block of 12 trials. The delay was set to 0, 2.5, 5, 10, and 20 s for each block.

Adjusting delay task (Fig. 8b). All animals were assigned a variable and an adjusting lever. A response on the variable lever resulted in the delivery of a liquid sucrose reinforcer (75 µl) after 0 or 20 s (probability = .5). A response on the adjusting lever resulted in the delivery of 75 µl of sucrose after *t* s, where *t* represents the adjusting delay. Variable lever choice decreased the adjusting delay by 10%, while adjusting lever choice increased the adjusting delay by 10%. Percentages were used instead of fixed

values due to the psychophysical relationship between the actual length of the delay length and perceived length of the delay. The adjusting delay was 5 s at the start of each session, and could not go above 22 s or below 0.1 s.

Adjusting magnitude task (Fig. 8c). All animals were assigned a variable and an adjusting lever. A response on the variable lever yielded 0 µl or 150 µl of sucrose (probability = .5) delivered immediately. A response on the adjusting lever immediately yielded a sucrose reinforcer with a magnitude of *m* µl, where *m* represents the adjusting magnitude. Variable lever choice increased the adjusting magnitude by 10%, while adjusting lever choice decreased the adjusting magnitude by 10%. The adjusting magnitude was 37.5 µl at the start of each session, and could not go above 165 µl or below 0.6 µl.

Animals' behavior had to reach stability in the task both before and after surgery. To test stability, data from the most recent 10 sessions were split into two sets of 5, and the average was taken for each of these sets. The data for each task were further split into five 12-trial blocks to accommodate the different delays of the discounting task. Behavior was considered stable when a 2 x 5 ANOVA (Set x Block) indicated no significant difference between the first and second set of sessions across each block of 12 trials for the dependent measure of interest (i.e. adjusting delay, adjusting magnitude, choices of delayed lever).

Surgery

After animals attained stable performance in their respective tasks (see previous paragraph), they underwent cranial surgery. Animals were anesthetized using 2-5% isoflurane during surgery and were administered subcutaneously 100,000 units of

penicillin to prevent infection and 5 mg/kg of carprofen as an analgesic. Bilateral 10-mm long 21-gauge cannulae were inserted with coordinates of either +3.7 mm anteroposterior (AP) from bregma, ±2.6 mm mediolateral (ML) from bregma, and -3.3 dorsoventral (DV) from the skull surface (lOFC) or +1.6 mm AP, ±1.8 mm ML, and -3.3 DV (AcbC). Metal stylets extending 0.5 mm beyond the tip of the cannulae were inserted to prevent clogging. On test days, lOFC injectors extended 1 mm beyond the tip of the cannulae, while AcbC injectors extended 3.5 mm beyond the tip of the cannulae. Injector tips were constructed of 32-gauge stainless steel, and the injectors were connected via polyethylene tubing to pumps with Hamilton 10 µl syringes. Animals were given a minimum of 5 days recovery post-surgery before resuming behavioral testing.

Drugs

The $GABA_B$ and $GABA_A$ agonists baclofen and muscimol were used to inactivate the lOFC and AcbC in this study. However, although the current study infers inactivation based upon previous work showing that these substances decrease neuronal activity (Olpe et al., 1988; van Duuren et al., 2007), it should be noted that the current study did not explicitly measure levels of neuronal activity.

Baclofen and muscimol (Sigma-Aldrich, St. Louis, MO, USA) were both separately dissolved in 0.9% saline at a concentration of 0.5 g/mL and stored in frozen aliquots. On each injection day, baclofen and muscimol were thawed and mixed together. For lOFC injections, rats were given 125 ng / 125 ng of baclofen/muscimol in 0.5 µl/hemisphere delivered over the course of 1 min 40 s (St. Onge & Floresco, 2010). For the AcbC, I initially planned to use the same concentration (250 ng/ µl) within a smaller volume (0.3 µl/hemisphere, yielding 75 ng / 75 ng baclofen/muscimol), as had

been done previously in an operant task (Ghods-Sharifi & Floresco, 2010). However, preliminary data showed that 75 ng / 75 ng baclofen/muscimol in the AcbC greatly reduced responding in all of the tasks (also see Figs. 19 and 20 from Appendix 3). Thus, rats were instead given 6.409 ng / 0.342 ng of baclofen/muscimol in 0.3 µl/hemisphere delivered over the course of 1 min. This dose did not suppress responding in the task, but has been shown to affect behavior in an operant task in a prior study (reinstatement for cocaine, McFarland & Kalivas, 2001). Injectors were left in the cannulae for 1 min following infusion to allow diffusion of the drugs.

Injections were administered on Tuesdays and Fridays. All animals initially received a saline injection to habituate them to the procedure. They then were administered 3 injections each of saline and baclofen/muscimol according to an alternating schedule (6 injections total). Half of the animals began with saline; the other half with baclofen/muscimol.

Histology

Following testing, animals were sacrificed by carbon dioxide exposure. Brains were removed and stored in 2% paraformaldehyde for 24 hours, followed by storage in 20% and then 30% sucrose/0.1% sodium azide. Sections (40 µm) were sliced on a cryostat, mounted on slides, and subsequently stained with thionin. These were then examined under a microscope and injector tip placements were mapped onto a rat atlas (Paxinos & Watson, 1998; see Fig. 9).

Data Analysis

The dependent measures for each of the three tasks were number of choices of the delayed lever, the adjusting delay, and the adjusting magnitude. All data were averaged

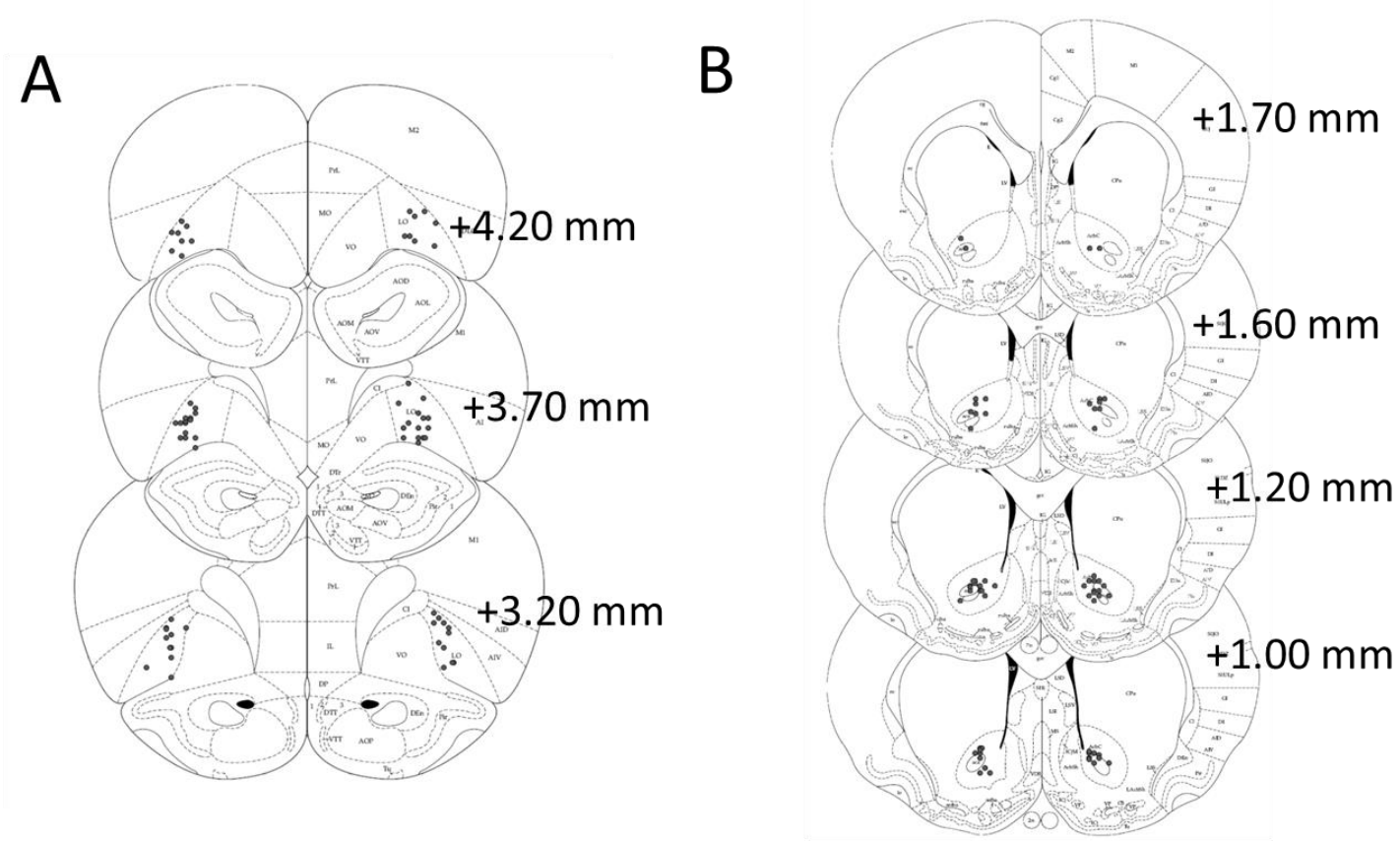


Fig. 9. Placement of injector tips in the lOFC **(A)** and AcbC **(B)**.

across the 3 injection sessions for each dose after removing the excluded data listed below. Data were subsequently organized into five 12-trial blocks to accommodate the different delays used in each 12-trial block in the discounting task. The data were analyzed with a 2 x 5 (Dose x Block) mixed-model ANOVA to compare saline with baclofen/muscimol (there were no effects of saline itself on the dependent measures, $Fs < 1.99$, $ps > 0.191$). Additionally, animals were divided into 'high' and low' groups to determine if baseline task performance modulated the effects of inactivation. To do this, the data were first averaged for each rat across the final 5 days before the injection phase began. Using these data, the average was calculated for all five blocks for each rat, and animals were grouped according to a median split. The injection data were analyzed for Groups with a 2 x 2 x 5 mixed-model ANOVA (Group x Dose x Block). Lastly, to evaluate rats' ability to complete the task, latency data, the number of trials completed in the task, and number of extraneous lever presses were analyzed using a 3 x 2 mixed-model ANOVA (Task x Dose), including data from sessions with uncompleted trials. Huynh-Feldt-corrected degrees of freedom were used wherever there were violations of sphericity and Bonferroni post hoc tests were used to compare effects at individual blocks.

Exclusions

Final group sizes were: AcbC: discounting task, n = 12; delay task, n = 10; magnitude task, n = 10. lOFC: discounting task, n = 11; delay task, n = 12; magnitude task, n = 11. Of the initial 96 animals, 10 animals died (discounting task, n = 2; delay task, n = 3; magnitude task, n = 5) and 16 animals were excluded due to poor cannulae placement (AcbC delay task, n = 2; lOFC discounting task, n = 4; lOFC delay task, n = 9;

lOFC magnitude task, n = 1). The first cohort contained equal numbers of animals for each task and brain region (n = 8 each). However, since some groups in the first cohort lost disproportionate numbers of animals, the second cohort was uneven (AcbC discounting task = 6, AcbC delay task = 6, AcbC magnitude task = 6, lOFC discounting task = 12, lOFC delay task = 13, lOFC magnitude task = 5). Nonetheless, there were no significant differences in the dependent variable of interest (e.g. adjusting delay, adjusting magnitude, choices of the delayed lever) between cohorts ($Fs < 3.30$, $ps > 0.103$).

Sessions on which animals did not respond for all 60 free choice trials (AcbC: 18 injections, 10.1%; lOFC: 41 injections, 19.9%) were removed from the analysis of the primary dependent variables because the missing data for the final blocks of trials in those sessions rendered them incomparable to completed sessions. Four animals were removed from the primary analysis because they did not complete all 60 trials on any session (AcbC discounting task, n = 1; lOFC discounting task, n = 3). For number of trials completed, response latency, and extraneous responses, two data sets were analyzed: One comprising data without incomplete sessions (identical to the method described above), and one comprising all data. The latter analysis is possible in this case because the data were not split into blocks.

Results.

AcbC inactivation significantly increased choice of the large reinforcer at long delays (Dose x Delay: $F(2.43,26.70) = 3.90$, $p = 0.026$; see Fig. 10a), suggesting that AcbC inactivation decreased delayed discounting at longer delays. Furthermore, this

effect was almost entirely driven by low discounters (Group x Dose: $F(1,10) = 5.53$, $p = 0.041$; Group x Dose x Delay: $F(2.37, 23.71) = 3.17$, $p = 0.053$). Simple effects analyses show that low discounters strongly decreased discounting after inactivation of the AcbC (Dose x Delay: $F(4,20) = 6.96$, $p = 0.001$) but that high discounters were unaffected (Dose x Delay: $F(4,20) = 0.33$, $p = 0.856$; see Fig. 11a,d).

AcbC inactivation did not alter adjusting delay or adjusting magnitude $F(1,9) = 0.68$, $p = 0.432$; $F(1,9) = 0.01$, $p = 0.910$; see Fig. 10).

lOFC inactivation did not alter delay discounting, adjusting delay, or adjusting magnitude (Discounting: $F(1,10) = 2.00$, $p = 0.188$; Adjusting Delay: $F(1,10) = 0.00$, $p = 0.960$; Adjusting Magnitude: $F(1,10) = 0.01$, $p = 0.918$; see Figs. 10,12).

AcbC inactivation did not affect the number of trials completed in the tasks (saline: 58.12 ± 0.67 trials completed, baclofen/muscimol: 58.37 ± 0.92 trials completed; $F(1,31) = 0.10$, $p = 0.756$). In contrast, lOFC inactivation decreased the number of trials completed in the tasks (saline: 58.89 ± 0.40 trials completed, baclofen/muscimol: 54.28 ± 1.46 trials completed; $F(1,34) = 12.07$, $p = 0.001$). This was likely caused by an increase in both nosepoke latency (saline: 5.35 ± 0.65 s, baclofen/muscimol: 7.72 ± 0.90 s; $F(1,34) = 7.72$, $p = 0.009$) and lever press latency (saline: 3.24 ± 0.53 s, baclofen/muscimol: 7.23 ± 2.14 s; $F(1,34) = 5.19$, $p = 0.029$). These effects on latency were diminished when only including data in which animals completed all 60 trials, although there was still a strong trend towards an increase in lever press latency (nosepoke latency, saline: 4.59 ± 0.61 s, baclofen/muscimol: 5.45 ± 0.61 s; $F(1,30) = 1.43$, $p = 0.241$; lever press latency, saline:

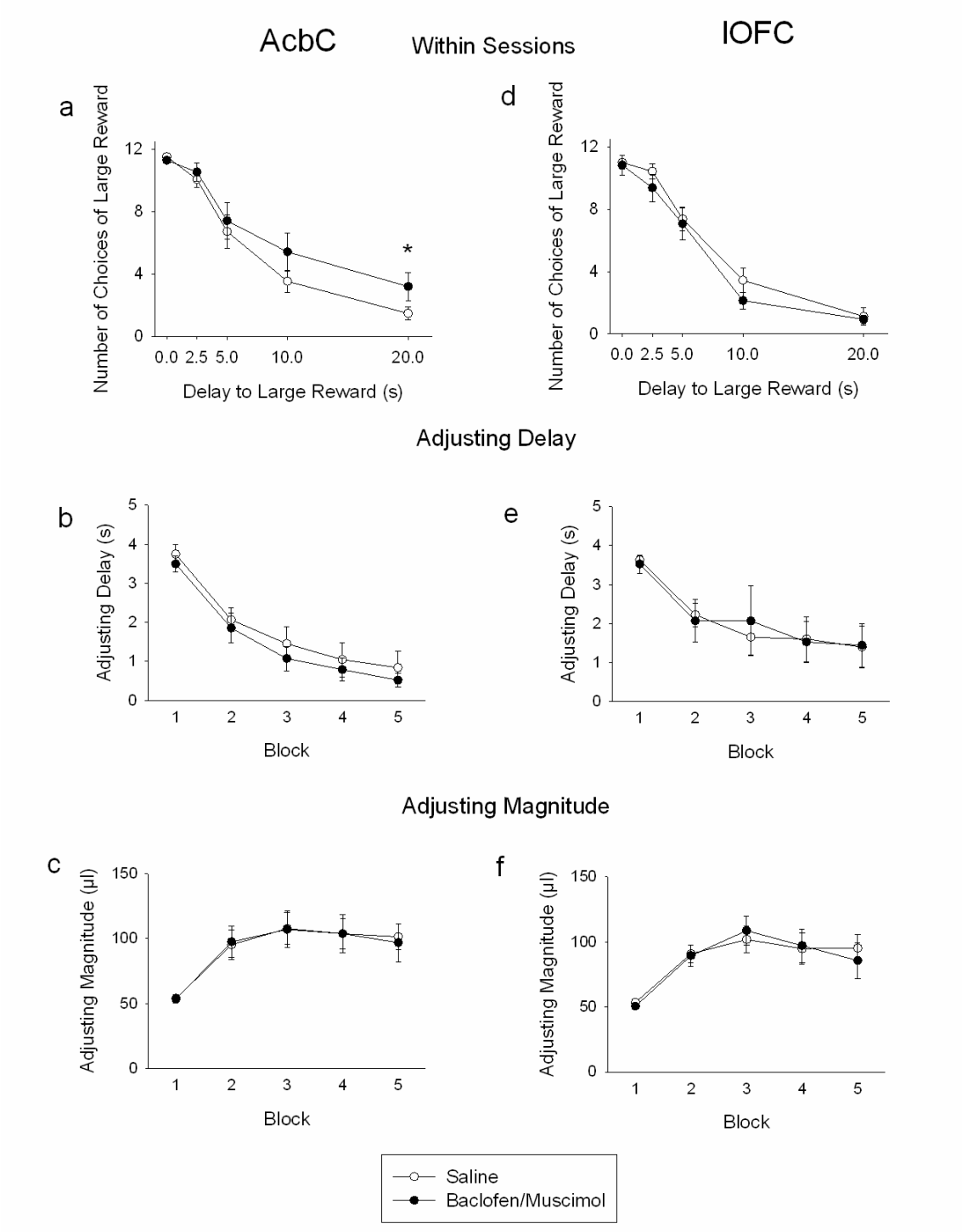

AcbC
Within Sessions
lOFC
a
d
Number of Choices of Large Reward
12
8
4
0
0.0 2.5 5.0
10.0
20.0
*
Delay to Large Reward (s)
Adjusting Delay
b
e
Adjusting Delay (s)
5
4
3
2
1
0
1 2 3 4 5
Block
Adjusting Magnitude
c
f
Adjusting Magnitude (µl)
150
100
50
0
Saline
Baclofen/Muscimol

Figure 10. Inactivation of the AcbC decreased delay discounting **(a)**, but had no effect on sensitivity to delay or magnitude **(b, c)**. Inactivation of the lOFC did not affect any measure **(d, e, f)**. * $p < 0.05$

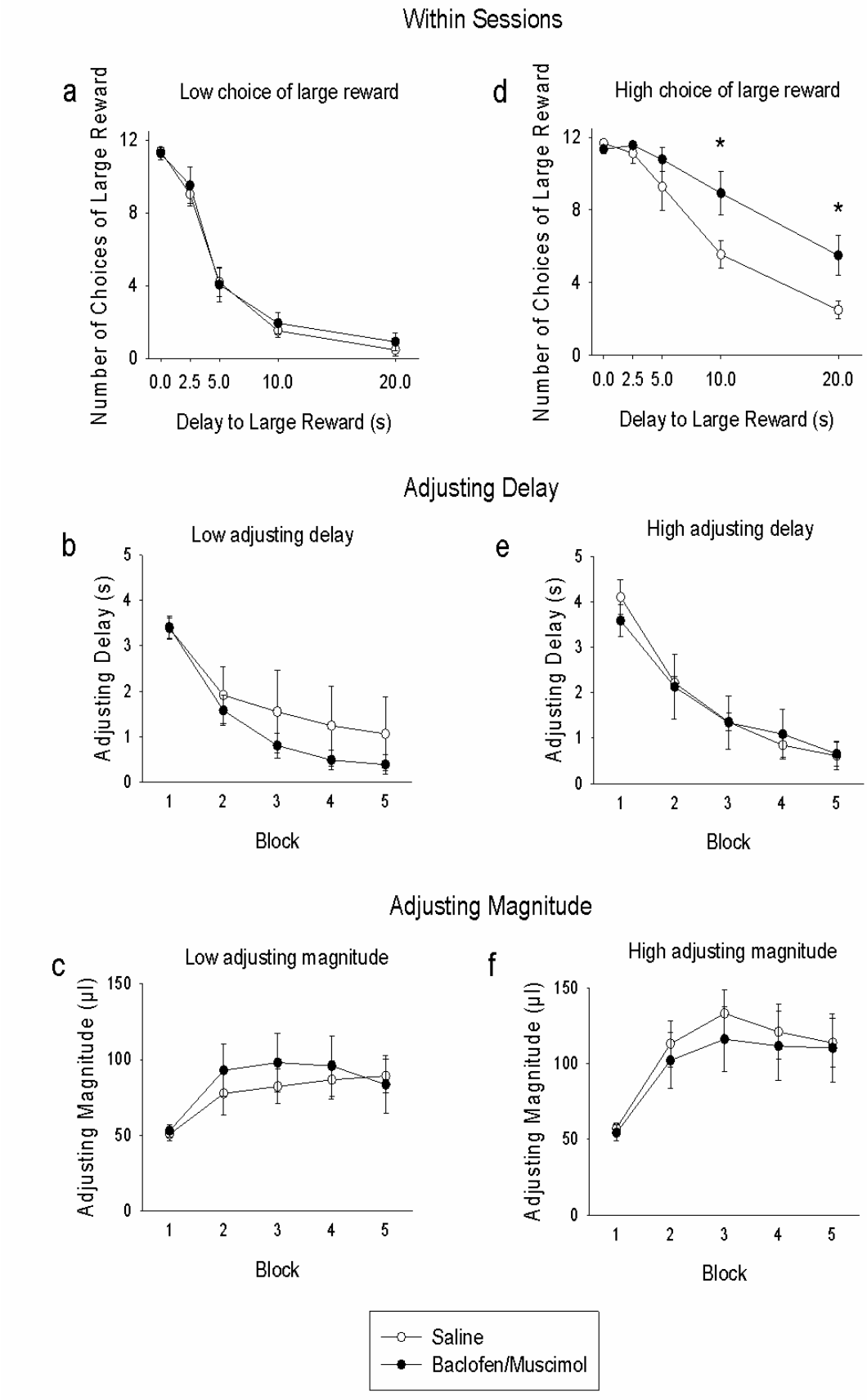
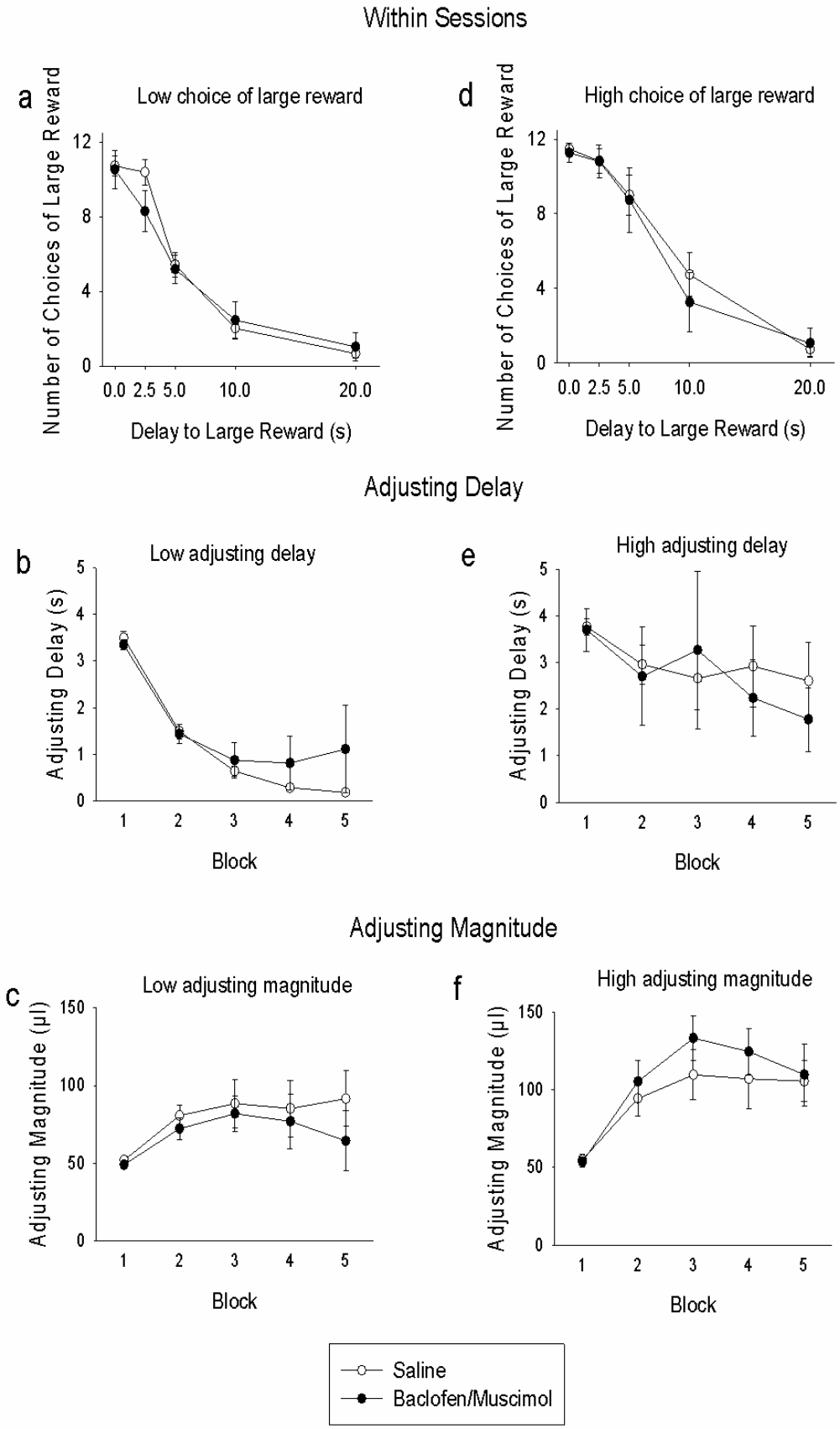

Within Sessions
a
Low choice of large reward
Number of Choices of Large Reward
12
8
4
0
0.0 2.5 5.0 10.0 20.0
Delay to Large Reward (s)
d
High choice of large reward
*
*
Adjusting Delay
b
Low adjusting delay
Adjusting Delay (s)
5
4
3
2
1
0
1 2 3 4 5
Block
e
High adjusting delay
Adjusting Magnitude
c
Low adjusting magnitude
Adjusting Magnitude (µl)
150
100
50
0
f
High adjusting magnitude
Saline
Baclofen/Muscimol

Figure 11. AcbC animals with few choices of the large reward (high discounting), low adjusting delay (high sensitivity to delay), and low adjusting magnitude (low sensitivity to magnitude) at baseline are on the left, and their counterparts are on the right. Inactivation of the AcbC decreased delay discounting in animals with high choices of the large reward (low discounting) **(d)**, but not in animals with low choices of the large reward (high discounting) **(a)**. No other effects were seen **(b, c, e, f)**. It should be noted that animals with low adjusting delay at baseline actually had higher adjusting delay after saline than animals with high adjusting delay at baseline **(b, e)**. This effect was entirely driven by one animal that had a very large adjusting delay after saline. However, removal of this animal from the analysis did not affect the results. * $p < 0.05$


Within Sessions
a
Low choice of large reward
Number of Choices of Large Reward
12
8
4
0
0.0 2.5 5.0
10.0
20.0
Delay to Large Reward (s)
d
High choice of large reward
Number of Choices of Large Reward
12
8
4
0
0.0 2.5 5.0
10.0
20.0
Delay to Large Reward (s)
Adjusting Delay
b
Low adjusting delay
Adjusting Delay (s)
5
4
3
2
1
0
1 2 3 4 5
Block
e
High adjusting delay
Adjusting Delay (s)
5
4
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2
1
0
1 2 3 4 5
Block
Adjusting Magnitude
c
Low adjusting magnitude
Adjusting Magnitude (µl)
150
100
50
0
1 2 3 4 5
Block
f
High adjusting magnitude
Adjusting Magnitude (µl)
150
100
50
0
1 2 3 4 5
Block
Saline
Baclofen/Muscimol

Figure 12. lOFC animals with few choices of the large reward (high discounting), low adjusting delay (high sensitivity to delay), and low adjusting magnitude (low sensitivity to magnitude) are on the left, and their counterparts are on the right. No effects of inactivation were seen **(a, b, c, d, e, f)**.

2.75 ± 0.26 s, baclofen/muscimol: 3.43 ± 0.42 s; $F(1,30) = 3.81$, $p = 0.060$). A similar increase in response latency was seen by Zeeb et al. (2010), although to a lesser degree.

Additionally, both AcbC and lOFC inactivation increased the number of extraneous lever presses performed throughout the session, although this effect was much stronger after lOFC inactivation (AcbC: saline: 64.31 ± 7.94 presses, baclofen/muscimol: 79.64 ± 9.05 presses; $F(1,31) = 5.72$, $p = 0.023$; lOFC: saline: 65.61 ± 8.81 presses, baclofen/muscomol: 110.42 ± 12.74 presses; $F(1,34) = 23.76$, $p < 0.001$). Notably, this effect persisted even when only including data in which animals completed all 60 trials (AcbC: saline: 63.22 ± 7.86 presses, baclofen/muscimol: 80.48 ± 9.31 presses; $F(1,30) = 6.88$, $p = 0.014$; lOFC: saline: 67.04 ± 9.39 presses, baclofen/muscomol: 118.41 ± 14.47 presses; $F(1,31) = 15.22$, $p < 0.001$). This suggests that the increase in response latency in lOFC rats is unlikely to be due to a decrease in motor function or motivation. Indeed, ability of lOFC inactivation to increase extraneous responses, but decrease the number of trials completed is very similar to that seen after lesion of the entire OFC (Chudasama et al., 2003).

Discussion.

The primary finding of interest was that inactivation of the AcbC decreased delay discounting, suggesting that the AcbC normally acts to promote impulsive choice. This result was surprising, given that the majority of studies have found that lesions of the AcbC *increase* delay discounting (Bezzina et al., 2007; Bezzina et al., 2008; Cardinal et al., 2001; da Costa Araújo et al., 2009; Galtress & Kirkpatrick, 2010; Pothuizen et al., 2005; but see Acheson et al., 2006). One notable difference between the current study and the others was the use of reversible inactivations rather than lesions. Importantly,

permanent loss of the AcbC may lead to compensation from other brain regions. Indeed, while AcbC lesions result in locomotor hyperactivity (Maldonado-Irizarry & Kelleyt, 1995; Parkinson et al., 1999), AcbC inactivations result in locomotor *hypo*activity (Fuchs et al., 2004; Ghods-Sharifi & Floresco, 2010). Furthermore, at least one study has shown a progressive increase in discounting across sessions for AcbC-lesioned animals, and has also demonstrated that these animals initially had a tendency towards *decreased* discounting compared to shams (Pothuizen et al., 2005). Future studies should investigate this possibility by examining the effect of AcbC lesions and inactivations within the same discounting experiment.

Although the current results are at odds with the lesion studies, they do have some support in the human literature. Early imaging studies have suggested that increased ventral striatal activity is associated with choosing the immediate reward (McClure et al., 2004, 2007). However, subsequent studies have suggested that ventral striatal activity is associated with the overall subjective value of the reward, be it delayed or not (Kable & Glimcher, 2007; Sripada et al., 2011). It may be that partially inactivating the AcbC decreases animals' ability to identify the subjective value of the reward, causing them to either choose randomly or to perseverate with their initial preference for the large reinforcer. The animals did not choose randomly in any of the tasks, ruling out one possibility. Additionally, although the decrease in delay discounting could be the result of perseverative responding for the large reinforcer, no similar perseverative result was seen in the other two tasks. Thus, at present the data from the current study seem to favor the ventral striatum's role in immediacy over the ventral striatum's role in subjective value.

AcbC inactivation significantly decreased delay discounting in a task requiring *both* sensitivity to delay and reinforcer magnitude, but it did not modulate sensitivity to delay or reinforcer magnitude in isolation. This suggests that manipulating the AcbC only affects behavior that synthesizes both delay and magnitude. Such a conclusion appears to conflict with previous results, which suggest that the AcbC is involved in sensitivity to delay *per se* (Bezzina et al., 2007; da Costa Araújo et al., 2010; Kheramin et al., 2002). However, the studies by Kheramin et al. (2002) and Bezzina et al. (2007) used tasks that manipulated both delay *and* magnitude, and had no explicit measure of delay sensitivity in isolation. On the other hand, the study by da Costa Araújo et al. (2010) used a task that independently assessed sensitivity to delay, but did not directly manipulate the AcbC. Although the authors found that exposure to the adjusting delay task increased c-fos counts in the AcbC, this does not establish a causal link between neuronal activity and behavioral output. Furthermore, there is some evidence that neurons differentially encode delay and magnitude depending on the task used. Roesch & Bryden (2011) noted that, when using tasks that *independently* manipulated delay and magnitude, many individual neurons in the ventral striatum encoded either delay or magnitude, but few encoded both. Conversely, in a study by Cai et al. (2011), wherein *both* delay and magnitude were manipulated within the same task, ventral striatal neurons that encoded the delay of the chosen reward were also highly likely to encode the magnitude of that reward. Thus, the results suggest that the firing patterns of neurons in the ventral striatum depended on whether the task included *both* delay and magnitude or only one of the two.

However, it is also possible that the procedural differences between the adjusting tasks and the discounting task were enough to obscure an effect on sensitivity to delay or magnitude in isolation. There were two main elements of the adjusting tasks that differed from the discounting task: the choice-dependent titrating design and the probabilistic component. However, there is little evidence that either of these would confound the experiments conducted here. A study using a titrating task found that lesions of the AcbC increased discounting (da Costa Araújo et al., 2009), so it seems unlikely that the titrating design would block an effect of AcbC inactivation. Additionally, although AcbC lesions have been shown to affect probability discounting (Cardinal & Howes, 2005), the evidence is mixed (Acheson et al., 2006) and inactivations of the AcbC have found no effect (Stopper & Floresco, 2011).

In addition to the general effect on discounting, inactivation of the AcbC predominantly decreased delay discounting in animals that had a low level of discounting to begin with. This may suggest that lower discounters have different AcbC physiology. Such individual differences in the physiological makeup of the nucleus accumbens have been associated with individual differences in a number of different behaviors, including both behavioral inhibition and the self-administration of cocaine (Dalley et al., 2007). Indeed, one study found that low discounters had more evoked dopamine released in the AcbC than high discounters (Diergaarde et al., 2008), although other studies found no relationship between levels of discounting and dopamine receptor mRNA in the AcbC (Loos et al., 2010a; Simon et al., 2013). Nonetheless, it is also possible that the AcbC is either regulating or being regulated by another brain region that plays a more direct role in the individual differences seen in discounting. For example, the two aforementioned

studies did find a relationship between delay discounting and levels of dopamine receptor mRNA in the medial prefrontal cortex (Loos et al., 2010a; Simon et al., 2013), which sends projections to the AcbC (Öngür & Price, 2000).

Inactivation of the lOFC did not affect delay discounting, which is in agreement with other studies using inactivation (Churchwell et al., 2009; Zeeb et al., 2010). However, there was no specific decrease in delay discounting for animals with high baseline discounting, as was reported in Zeeb et al. (2010). Nonetheless, the effect in Zeeb et al.'s study was fairly small and occurred primarily at the 45 s time interval. Thus, a larger sample size and longer delays may have been required to replicate the effect, although the small effect size (partial $\eta^2 = .107$) may suggest otherwise.

In conclusion, these results show that, contrary to most lesion studies, AcbC inactivation decreased delay discounting. I suggest that prior studies using lesions may have seen a compensatory effect, and that temporary inactivation of the AcbC may more accurately model the role of the AcbC in real time. In addition, these results show that the AcbC did not modulate sensitivity to delay or magnitude when the two were independently assessed, which may indicate that the AcbC only modulates behavior synthesizing both delay *and* magnitude. The effect of AcbC inactivation on discounting was greatest in animals with low levels of discounting at baseline, and I believe that future studies should examine the role that the AcbC and its efferent/afferent projections play in these individual differences. Lastly, the data suggest that the lOFC does not play a role in any of the aforementioned processes. In total, these findings suggest a complex role for the AcbC in discounting, and support existing evidence that temporary inactivation of the lOFC has no effect on discounting.

Chapter 4. General Discussion.

The purpose of this dissertation was to understand the effect that systemic and intracranial pharmacological manipulations had on delay discounting and its component processes: sensitivity to delay and sensitivity to magnitude (see Fig. 2). The primary outcome of this work was that acute administration of these drugs had no effect on measures of either sensitivity to delay or magnitude. Several additional findings were also reported. Most notably, the data suggest that high sensitivity to delay predicts low sensitivity to ethanol's behaviorally suppressant effects, that AcbC inactivation decreases delay discounting, and that the latter effect is much stronger in animals that have low delay discounting at baseline. Each of these findings is discussed in more detail in the following pages.

Effect of acute pharmacological manipulations on sensitivity to delay and magnitude

No pharmacological manipulations, whether systemic or intracranial, had an effect on either adjusting delay or magnitude values, which were the primary dependent variables of the adjusting delay and magnitude tasks. The simplest explanation for this finding is that each of these acute pharmacological manipulations does not impact sensitivity to delay or sensitivity to magnitude. At first glance, this interpretation does not seem to fit with existing literature because each of the manipulations used in this dissertation have been shown to alter delay discounting. Ethanol increases discounting (Evenden and Ryan, 1999; Hellemans et al., 2005; Olmstead et al., 2006; Poulos et al., 1998; Tomie et al., 1998; but see Wilhelm and Mitchell, 2012), methamphetamine decreases discounting (Richards et al., 1999), lOFC inactivation decreases discounting

(Zeeb et al., 2010, only in animals with high baseline discounting; but see Churchwell et al., 2009), and AcbC inactivation was shown to decrease discounting in Chapter 3.

However, an important difference between the adjusting tasks used in this dissertation and the more commonly used delay discounting tasks is that the latter test sensitivity to delay and magnitude *simultaneously*. Thus, there may be a crucial difference between tasks assessing both delay and magnitude sensitivity, and those only assessing one of the two. This difference may be in the different neural correlates used for either task. *In vivo* electrophysiological studies have indirectly hinted at this possibility, showing that a high proportion of sampled neurons in the ventral striatum will encode the value of both delays and magnitudes in a task requiring sensitivity to both delay and magnitude (Cai et al., 2011), but not in two tasks that separately require sensitivity to only one of the two (Roesch & Bryden, 2011). However, the evidence at present is too scant to provide any firm conclusions.

It is important to recognize another possibility: that the procedural differences between tasks simultaneously assessing delay and magnitude (i.e. standard delay discounting tasks) and the tasks I used to assess them independently (i.e. the adjusting tasks) were responsible for the null results seen in the current study. As mentioned in Chapter 3, the primary differences between the delay discounting task and the adjusting tasks used in this study were the adjusting and probabilistic components employed in the latter. These differences are explicitly shown in Fig. 8. The delay discounting task (Fig. 8A) does not have the adjusting component (left choice in Fig. 8B,C) or the variable (i.e. probabilistic) component (right choice in Fig. 8B,C). Two of the manipulations used in this dissertation have been previously shown to affect behavior in adjusting delay

discounting tasks (methamphetamine: Richards et al., 1999; AcbC lesion: da Costa Araújo et al., 2009), demonstrating that these manipulations can affect delay discounting in an adjusting procedure. Thus, it seems unlikely that the adjusting component on its own could be responsible for the different results.

However, it may be that these manipulations interacted with the probabilistic component of the task in a way that obscured their effect on sensitivity to magnitude and/or delay. Some of the manipulations have been shown to affect probability discounting (OFC lesion: Mobini et al., 2002; AcbC lesion: Cardinal & Howes, 2005). Additionally, although methamphetamine has not been tested in probability discounting, the related drug amphetamine affects probability discounting (St. Onge et al., 2010). Indeed, ethanol is the only manipulation which has been clearly shown to not affect probability discounting (Mitchell et al., 2011). In spite of this, there is good reason to believe that these manipulations were unlikely to interact with the probabilistic component of the tasks. First, AcbC *inactivations* have no effect on probability discounting (Stopper & Floresco, 2011). Additionally, both OFC lesions and amphetamine have no effect on probability discounting when the probability is 50%, as was used in the adjusting tasks in this dissertation (Mobini et al., 2002; St. Onge et al., 2010).

When viewed in isolation, it seems unlikely that the adjusting or probabilistic components of the task were responsible for the discrepant results found. Nonetheless, it is possible that the combination of the two could have interfered with any effects on sensitivity to delay or magnitude. This could be especially true if the manipulations themselves had a fairly small effect on sensitivity to delay or magnitude, in which case

even the slightest interference from the adjusting or probabilistic components could obscure an effect.

In order to determine the role of procedural differences in the discrepant results found for adjusting and delay discounting tasks, an important future step would be elucidating the relationship between these tasks. This could be accomplished by first training animals in one task, then training them on the other, and then correlating their behavior in the two tasks. If, for example, adjusting delay correlated with steepness of the discounting curve, and adjusting magnitude correlated with bias for the immediate reward when there was no delay, then it would be plausible to argue that these tasks measure some of the same things (although it may not be so simple; see Appendix 1 regarding Killeen's (2009) additive-utility model of delay discounting). Indeed, similar studies have already been shown to correlate different delay discounting task variants (Green et al., 2007; Wilhelm et al., in prep, although the correlation was not significant, $p = .07$). If the two tasks are not related, it may be useful to isolate which elements are crucial in differentiating them (adjusting component, probabilistic component, etc.).

In summary, it was not feasible to form a clear conclusion regarding whether the pharmacological manipulations used in this study were able to alter sensitivity to delay or magnitude. While the data suggest that none of the manipulations affect sensitivity to delay or magnitude, they conflict with much of the existing literature (ethanol: Evenden and Ryan, 1999; Hellemans et al., 2005; Olmstead et al., 2006; Poulos et al., 1998; Tomie et al., 1998; but see Wilhelm and Mitchell, 2012; methamphetamine: Richards et al., 1999; lOFC inactivation: Zeeb et al., 2010, only in animals with high baseline discounting; but see Churchwell et al., 2009; AcbC inactivation: Chapter 3). Therefore,

while one conclusion is that these manipulations had no effect on sensitivity to delay and magnitude, it cannot be entirely ruled out that other factors limited the ability to observe these effects. Future studies, such as those suggested above, should be conducted to investigate the role of these other factors.

Ability of sensitivity to delay or magnitude to predict the behaviorally suppressant effects of acute manipulations

In addition to my primary research question, I wanted to know if behavior in any of the tasks could predict ethanol's ability to suppress behavior in any of the tasks. As reported in Chapter 2, animals with low adjusting delay were less affected by ethanol's suppressant effects. This finding may provide a link between some of the existing ethanol literature regarding delay discounting, ethanol consumption, and ethanol's suppressant effects on behavior. For example, rats selectively bred for high alcohol consumption exhibit less ethanol-induced suppression of locomotor activity than their low-consuming counterparts (Rodd et al., 2004; Waller et al., 1986). Additionally, animals that consume high amounts of ethanol or that have been selectively bred to consume high amounts of ethanol have higher drug-naïve delay discounting (Oberlin and Grahame, 2009; Poulos et al., 1995; Wilhelm and Mitchell, 2008; but see Diergaarde et al., 2012; Wilhelm et al., 2007; Wilhelm and Mitchell, 2012). Thus, it may be that high sensitivity to delay, low sensitivity to ethanol's suppressant effects, and high ethanol consumption are in part driven by the same underlying processes.

To test the hypothesized link between these three behaviors, a study could determine the relationship between adjusting delay and level of ethanol consumption. If low adjusting delay (high sensitivity to delay) predicted high ethanol consumption, it would support the existence of an underlying process driving increased delay

discounting, decreased sensitivity to the behaviorally suppressant effects of ethanol, and increased consumption of ethanol. Additional support would be added if performance in a standard delay discounting task also predicted behavioral suppression of responding. Furthermore, this would show some support for the notion that both adjusting delay tasks and delay discounting tasks measure sensitivity to delay.

Although there was a relationship between sensitivity to delay and ethanol's suppressant effects, it is difficult to draw clear conclusions from the data gathered for the other manipulations. This is primarily because the other studies did not use a dose that, like 0.9 g/kg ethanol, yielded a large degree of variability in behavioral suppression. The doses of methamphetamine, AcbC baclofen/muscimol, and lOFC baclofen/muscimol that were used had small or nonexistent suppressant effects. While more time could have been spent attempting to locate an ideal dose that maximized variability, this would have taken time away from focusing on the primary research question, and more importantly, would have required several additional microinjections into the AcbC and lOFC. This in turn would likely have increased brain damage and thus impaired the ability to detect placement of the injectors. However, future studies could investigate the relationships between sensitivity to delay/magnitude and behavioral suppression elicited by these other manipulations. This would be most feasible with AcbC inactivation, which was shown to have strong suppressant effects on behavior at high enough doses (Appendix 3). Indeed, the behaviorally suppressant effects of 64.09 ng / 3.42 ng baclofen/muscimol (second highest dose used in Appendix 3) varied widely among animals, suggesting that this would be a good dose to examine initially.

Effect of acute manipulations on delay discounting

The experiment in Chapter 3 included a commonly used delay discounting task. Based on the previous literature (Bezzina et al., 2007; Bezzina et al., 2008; Cardinal et al., 2001; da Costa Araújo et al., 2009; Galtress & Kirkpatrick, 2010; Pothuizen et al., 2005; but see Acheson et al., 2006), I anticipated that AcbC inactivation would increase delay discounting. I had a more open hypothesis regarding the effect of lOFC inactivation, based on the mixed literature (Abela et al., 2013; Finger et al., 2011; Jo et al., 2013; Kheramin et al., 2002; Kheramin et al., 2003; Mariano et al., 2009; Mobini et al., 2002; Rudebeck et al., 2006; Winstanley et al., 2004).

AcbC inactivation decreased delay discounting, which is the opposite finding of reported literature that used excitotoxic lesions (Bezzina et al., 2007; Bezzina et al., 2008; Cardinal et al., 2001; da Costa Araújo et al., 2009; Galtress & Kirkpatrick, 2010; Pothuizen et al., 2005). However, it is important to note that at least one study using excitotoxic lesions did find that lesions decreased delay discounting (Acheson et al., 2006). Additionally, inconsistent lesion results are not unheard of in the discounting literature (cf. the orbitofrontal cortex discounting literature previously referenced in this dissertation).

Nonetheless, the effect of AcbC inactivation was the opposite of the majority of the literature. This difference may be due to lesions eliciting compensatory mechanisms that result in an increase in delay discounting. Indeed, AcbC lesions increase locomotor activity, while AcbC inactivations decrease locomotor activity (lesions: Maldonado-Irizarry & Kelleyt, 1995; Parkinson et al., 1999, inactivations: Fuchs et al., 2004; Ghods-Sharifi & Floresco, 2010; Appendix 3). Nonetheless, the claim of compensatory

mechanisms would be better supported if behavior was compared for two groups of lesioned and inactivated animals simultaneously in the same delay discounting task. Further support would be garnered by following the day-by-day progress of the lesioned animals in the delay discounting task to determine if they changed their rate of discounting over time (as was seen in Pothuizen et al., 2005). Such a finding could have a few possible causes. One would be that lesion of the AcbC led to a gradual change in the function of the rest of the brain, leading to the gradual change in delay discounting seen. An alternative would be that lesion of the AcbC led to a rapid change in the function of the rest of the brain, but that the change in brain physiology only affects behavior incrementally. Such a question could be answered by testing Acb-lesioned animals at different time points after they were lesioned.

It is important to note that the low dose used likely only resulted in a *partial* inactivation of the AcbC, because higher doses decreased responding in the task. Thus, it may be that "partial lesions" would have led to results similar to mine. Additionally, different GABA receptor subtypes have varying degrees of affinity for GABA agonists (Farrant & Nusser, 2005). Therefore, it may be that the low dose preferentially activated a subset of these receptors, which may in turn have yielded a different firing pattern than would be expected from a pure inactivation. Future studies could test this through the use of inactivating agents that act through voltage-gated sodium channels rather than GABA receptors (such as lidocaine or tetrodotoxin), but the duration of the effect of these drugs would need to be considered when designing the study (effects of lidocaine diminish after 30 min, Martin, 1991; effects of tetrodotoxin can last for up to 2 days, Freund, 2010).

Caveats notwithstanding, the data as presented may have implications for interpretation of some of the human literature. Most notably, these results support data presented by McClure et al. (2004, 2007) that show heightened ventral striatal activity during decisions involving immediate rewards, suggesting that ventral striatal activity promotes heightened delay discounting. Equally importantly, the data do *not* support other human research that shows that heightened ventral striatal activity correlates with the subjective value of the reward, regardless of the presence of immediate rewards (Kable & Glimcher, 2007; Sripada et al., 2011).

Inactivation of the lOFC had no effect on delay discounting. This contrasted with the results found in some studies (Kheramin et al., 2002; Kheramin et al., 2003; Mobini et al., 2002; Rudebeck et al., 2006; Winstanley et al., 2004), but replicated those found in others (Abela et al., 2013; Finger et al., 2011; Jo et al., 2013; Mariano et al., 2009). Importantly, this finding replicated previous studies using OFC *inactivations* (Churchwell et al., 2009; Zeeb et al., 2010).

Ability of sensitivity to delay or magnitude to predict the effects of acute manipulations on sensitivity to delay or magnitude

Baseline task performance did not appear to predict any effects of ethanol or methamphetamine on either adjusting delay or adjusting magnitude, although the small size of the "high" and "low" groups (n = 4 for each group) makes it difficult to come to any strong conclusions. Contrary to expectations, lOFC inactivation also had no effect on any dependent measure, regardless of baseline performance in the task. This failed to replicate findings by Zeeb et al. (2010), but the small sample size and procedural differences should be taken into account when comparing these two studies. Zeeb et al. (2010) had 8 animals per high and low group (compared to 5 animals per group in used in

Chapter 3). Furthermore, the effect seen in Zeeb et al. (2010) was small, and occurred predominantly at the 40 s delay. Since the longest delay used in Chapter 3 was only 20 s, a longer delay may have been required in Chapter 3 to see the effect.

In stark contrast to the above, the effects of AcbC inactivation on delay discounting appeared to be strongly modulated by drug-naïve levels of delay discounting. AcbC inactivation greatly decreased inactivation in rats that had low levels of discounting to begin with, but had no effect on animals that had high levels of discounting. Thus, AcbC activity appears to play a different role in discounting for these two subgroups. In total, these results suggest that high and low discounters may have different AcbC physiology (as evidenced by Diergaarde et al., 2008).

To further test this claim, one could investigate the differences in AcbC physiology between high and low discounters. Given that high and low discounters differ in the amount of evoked dopamine released into the AcbC (Diergaarde et al., 2008), a good place to start would be to investigate the role that AcbC dopamine plays in delay discounting. Indeed, studies have already shown that systemic administration of dopamine agonists and antagonists can alter discounting (van Gaalen et al., 2006; Wade et al., 2000). Microinfusion of these agents into the AcbC could determine if AcbC dopamine is the mediator of these effects.

Additionally, several other brain regions are prime candidates for involvement in the aforementioned effects. The ventral tegmental area is the primary source of dopaminergic projections to the AcbC (Hyman et al., 2006), and has not been studied within the context of animal models of delay discounting. Another brain region, the medial prefrontal cortex, also sends direct projections to the AcbC (Berendse et al.,

1992). Notably, levels of dopamine receptor mRNA in the medial prefrontal cortex differ for high and low discounters (Loos et al., 2010a; Simon et al., 2013). Thus, differences in medial prefrontal cortex physiology could be influencing the individual differences seen in delay discounting after AcbC inactivation.

Effect of lOFC inactivation on task performance

Although lOFC inactivation had no effect on sensitivity to delay or magnitude, it did affect several other aspects of behavior. Specifically, lOFC inactivation decreased the number of trials completed in the task, increased latency to respond, and increased extraneous responses. This pattern of responses is very similar to that seen after lesion of the entire OFC in the 5-choice serial reaction time task, which measures attention, behavioral disinhibition, and perseverative responding (Chudasama et al., 2003). Lesions of the OFC increased premature responding (the measure of behavioral disinhibition) and perseverative responding, similar to the increase in extraneous responding seen in Chapter 3. OFC lesions also increased the number of omitted trials, analogous to the decreased number of trials completed seen in Chapter 3.

The results in Chapter 3 suggest that the effects seen in Chudasama et al. (2003) may have been driven by the lesion of the lOFC in particular. However, in order to determine if the effects of lOFC inactivation are the same as those seen after lesion of the entire OFC, one would have examine the effect of lOFC inactivation on performance in the 5-choice serial reaction time task. Based on the results listed above, one might expect to replicate the previous findings found with OFC lesion: An increase in premature and perseverative responding and a decrease in the number of trials completed (Chudasama et al., 2003). However, such a result is not easily interpretable. Although Chudasama et al.

(2003) suggested that this behavior may have been the result of animals having difficulty orienting to the stimulus, this explanation does not explain the increase in extraneous responses seen, nor does it explain why animals would have such difficulty with orientation. In the end, it may be best to visually record the behavior of animals and/or analyze the microstructure of the behavioral responses in the tasks to determine the nature of the behavioral changes induced by lOFC inactivation.

Effect of acute manipulations on locomotor activity

Lastly, I conducted several experiments showing that both ethanol and AcbC inactivation decreased locomotor activity (see Appendix 2, second experiment and Appendix 3, first experiment). Notably, ethanol only decreased vertical activity (although higher doses would have decreased horizontal activity as well, Duncan et al., 2000), and AcbC inactivation decreased vertical activity at lower doses than it did horizontal activity. These findings have several implications.

First, the findings clearly demonstrate the importance of gathering data for both vertical and horizontal activity. Many locomotor studies using rats only gather horizontal data. For example, there are several studies examining the effect of AcbC inactivation on horizontal activity, but until now no study that I know of has examined the effects on vertical activity (most studies did not have photobeams high enough to assess vertical activity; Fuchs et al., 2004, 2008; Floresco et al., 2008; Ghods-Sharifi & Floresco, 2010; McFarland & Kalivas, 2001; Stopper & Floresco, 2011).

The fact that both drugs affected vertical activity at lower doses than they did horizontal activity has implications for the interpretation of studies using either of these two drugs. For example, McFarland & Kalivas (2001) showed that 64.09 ng / 3.42 ng of

baclofen/muscimol decreased responses during cue-induced reinstatement for cocaine, but had no effect on horizontal activity. Therefore, they concluded that AcbC inactivation specifically blocked cue-induced cocaine reinstatement without affecting general locomotion/motivation. However, without the vertical activity data it is hard to verify this supposition.

The finding that ethanol decreased vertical activity at a dose that did not reduce horizontal activity may have further implications. Because vertical activity requires the animal to rear up on its hind legs, whereas horizontal activity only requires the animal to walk, vertical activity likely requires more effort. Therefore, ethanol may increase *effort* discounting, that is, it may increase the choice of a small reinforcer requiring less effort over that of a large reinforcer requiring more effort. Inactivation of the AcbC has been shown to increase effort discounting (Ghods-Sharifi & Floresco, 2010), and Appendix 3 showed that it also decreased vertical activity at lower doses than it did horizontal activity, similar to ethanol. Additionally, ethanol has been shown to decrease breakpoints attained during a progressive ratio task using food as the reinforcer (Wenger & Hall, 2010), which may suggest a decrease in effortful responding. Thus, there is a good chance that ethanol could increase effort discounting, and further research should investigate this possibility.

Conclusion

This dissertation found that ethanol, methamphetamine, lOFC inactivation, and AcbC inactivation each had no effect on measures of sensitivity to delay or magnitude. These findings are at odds with the majority of the delay discounting literature, and there are at least two possible explanations for this outcome. First, it may be that tasks

measuring sensitivity to delay and magnitude simultaneously require different neural substrates than those measuring sensitivity to delay and magnitude separately. Second, it may be that the procedural differences between delay discounting tasks and the adjusting tasks were sufficient to result in the discrepant findings.

This work also included several other findings. Baseline levels of sensitivity to delay were positively correlated with resistance to the behaviorally suppressant effects of ethanol, as well as negatively correlated with the effects of AcbC inactivation, and AcbC inactivation decreased delay discounting. The first finding appears to fit in with existing literature and may provide a link between delay discounting and the behaviorally suppressant effects of ethanol. The latter finding contrasts with much of the animal literature, but may suggest a crucial distinction between AcbC lesion and AcbC inactivation.

In total, this dissertation suggests that more work needs to be done to determine what the delay discounting tasks and adjusting tasks are measuring. Additional studies should also follow up on the findings with ethanol and AcbC inactivation. Future studies examining these relationships should give serious consideration to the elements of study design (e.g. length of delay, probabilistic elements, etc.), as subtle differences in design may yield large differences in outcome.

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Appendix 1. Mathematical and theoretical background for the adjusting delay and adjusting magnitude tasks.

The background for both the adjusting delay and adjusting magnitude tasks that I used (Chapters 2 and 3) is provided in previous studies (Mazur, 1984; da Costa Araújo et al., 2010). However, I have not provided a mathematical rationale for why an increase in sensitivity to delay would result in choices that cause a decrease in adjusting delay, or why a decrease in sensitivity to magnitude would result in choices that cause a decrease in adjusting magnitude. In this Appendix I will briefly provide this mathematical rationale and describe how these adjustments are associated theoretically with the subjective value and the utility of the alternative.

Sensitivity to delay

Several attempts have been made to mathematically characterize delay discounting (e.g. Ainslie, 1975; Logue, 1984; Killeen, 2009). One of the most widely used models is a hyperbolic function:

$$V_d = \frac{V_0}{1 + kd} \qquad (1)$$

Where V_0 represents the subjective value of a reinforcer after no delay, V_d represents the discounted value of a reinforcer after delay d, and k represents the individual's discounting rate, which is the measure of that individual's sensitivity to delay. This model more accurately describes empirical data than an exponential decay model (Ainslie, 1975; Mazur, 1987) and predicts certain phenomena such as preference

reversals[3] (Monterosso & Ainslie, 2007). Thus, I have used this model as a basis for my subsequent models.

One experimental method used to isolate delay from magnitude was developed by Mazur (1984), who designed the adjusting delay task used in the current studies (see Chapter 2 Methods for a full description of this task). For this task, Mazur found that the following equation fit empirical data as a measure of the value of a variable delay reinforcement contingency that contained multiple different delays (such as was used for the "variable" lever in the adjusting delay task):

$$V_{d'} = \sum_{v=1}^{n} p_v \left(\frac{V_0}{1 + k d_v} \right) \qquad (2)$$

As with Equation 1, V_0 represents the value of a reinforcer after no delay and k represents the individual's discounting rate. This equation can also account for multiple delays (specifically, n delays). The equation multiplies the discounted value at each delay (inside the parentheses) with the probability of that delay's occurrence (p_v), and then sums those values together. Thus, $V_{d'}$ is the weighted average of the discounted value for each delay.

To yield an empirically measurable value for $V_{d'}$, Mazur contrasted the variable delay reinforcement contingency, under which reinforcers were delivered following one of two distinctly different delays, with an adjusting delay reinforcement contingency under which the delivery delay changed incrementally according to the animals' pattern

[3] Preference reversals occur when an individual switches their preference from a large, later reward to a small sooner reward as they approach the smaller reward in time. For example, many people would prefer $100 after 10 years to $90 after 9 years. However, 9 years later many would switch their preference to $90 today vs $100 in a year.

of responding (like that used for the adjusting lever in the adjusting delay task in Chapter 2 and 3). Because the nature of the change on a trial-by-trial basis was relatively small, Mazur considered the subjective value of this response alternative to be relatively "fixed", and determined its value using Equation 1. Thus, when the value of the adjusting delay became equivalent to that of the combined value of the variable delays, the animal became indifferent between the two alternatives, which in turn caused the delay associated with the adjusting schedule to vacillate between a stable range of values. The following equation can be used to describe this relationship:

$$\frac{V_0}{1 + kd_a} = \sum_{v=1}^{n} p_v \left(\frac{V_0}{1 + kd_v}\right) \qquad (3)$$

Note that d_a in this equation refers to the adjusting delay and that V_0 is the same on both sides of the equations because the same amount of reinforcement is delivered for each contingency. Additionally, if we only use two delays with equal probability (p = ½), as was used in the adjusting delay task, and if we also let V_0 equal 1, which is justified because it is a constant of equal value for either lever, we can arrive at the following equation:

$$\frac{1}{1 + kd_a} = (0.5)\left(\frac{1}{1 + kd_1}\right) + (0.5)\left(\frac{1}{1 + kd_2}\right) \qquad (4)$$

Using this equation, we can determine an animal's sensitivity to delay (k), given the two discrete delays that we subject the animal to (d_1 and d_2) as well as the adjusting

delay at indifference (d_a). More importantly, we can also predict how a change in k will result in a change in d_a. For example, if we assign 2 s and 18 s to d_1 and d_2, as was done for the adjusting delay task used in Chapter 2, we would have the following equation:

$$\frac{1}{1+kd_a} = (0.5)\left(\frac{1}{1+k(2)}\right) + (0.5)\left(\frac{1}{1+k(18)}\right) \qquad (5)$$

We can then solve for the relationship between d_a and k:

$$d_a = \frac{36k+10}{10k+1} \qquad (6)$$

Within the constraints of $k \geq 0$ and $d_a \geq 0$, an increase in k will result in a decrease in d_a (see Fig. 13a). In other words, according to this equation, an increase in sensitivity to delay will result in a decrease in adjusting delay.

Sensitivity to magnitude.

While much work has gone into modeling sensitivity to delay, much less has gone into modeling sensitivity to magnitude. Because of this, I will provide two different models of magnitude sensitivity. Extrapolation from both models leads to the same conclusion. The first model was designed by the same group that developed the adjusting magnitude task (Ho et al., 1999):

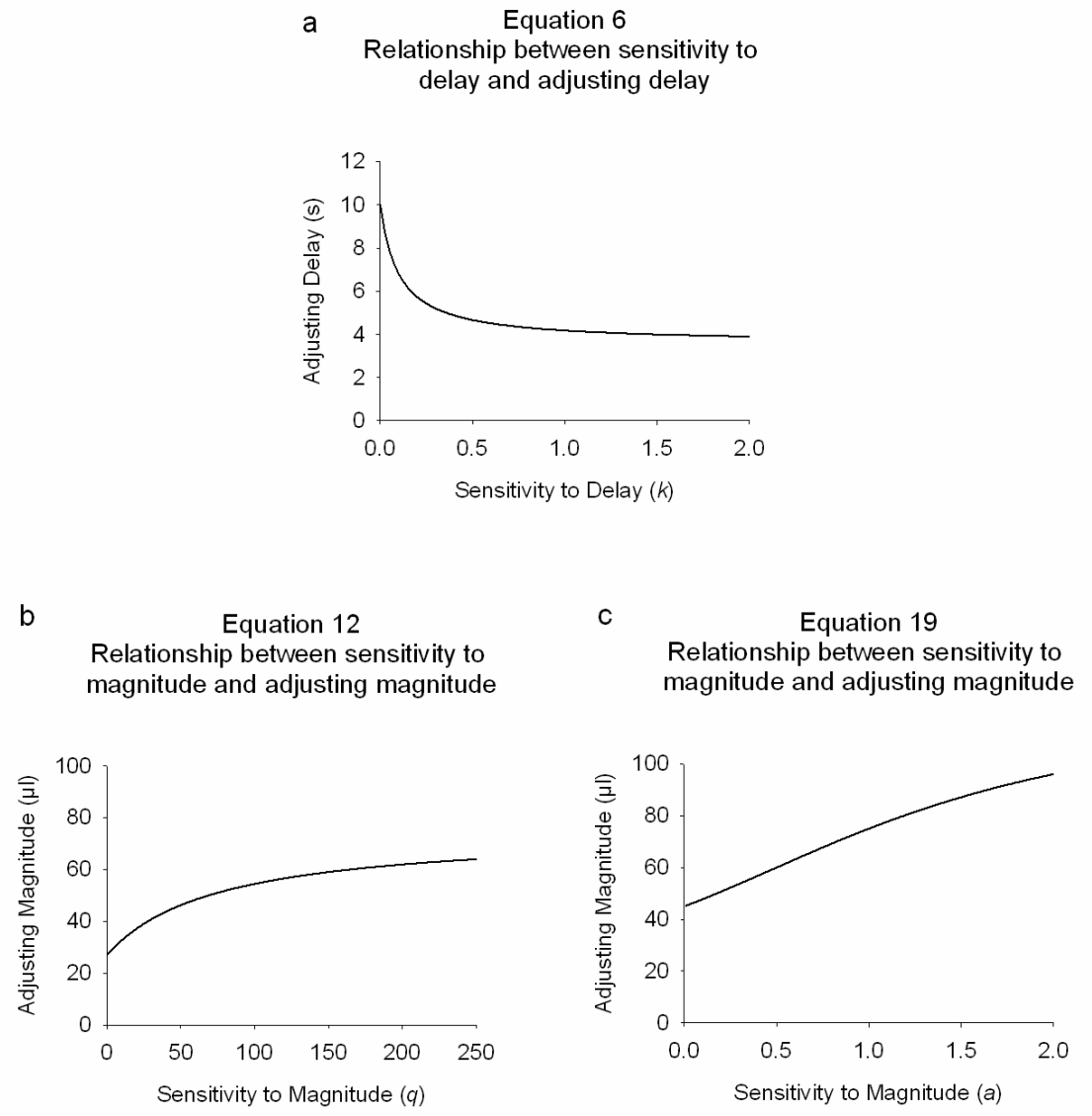

Figure 13. Mathematical relationships between sensitivity and adjusting values. **a)** The relationship between sensitivity to delay and adjusting delay based on the equation derived from the hyperbolic discounting function. **b)** The relationship between sensitivity to magnitude and adjusting magnitude based on the equation derived from Ho et al. (1999). **c)** The relationship between sensitivity to magnitude and adjusting magnitude based on the equation derived from Killeen (2009).

$$V_m = \frac{1}{1 + q/m} \tag{7}$$

The equation is similarly structured to the hyperbolic discounting function, with some key differences. First, there is no V_0, because this equation assesses sensitivity to magnitude rather than delay. In fact, the authors intended that Equation 7 be substituted in for V_0 in Equation 1, so that sensitivity to delay and magnitude could be calculated together. The second difference from Equation 1 is that this equation uses *q* and *m* rather than *k* and *d*. *m* represents the magnitude of the reward, and *q* represents the magnitude discounting rate, or sensitivity to magnitude.

Equation 7 can follow the same process that Equation 1 went through for delay. First, we modify the equation for the variable reinforcement contingency (as with Equation 2):

$$V_{m'} = \sum_{v=1}^{n} p_v \left(\frac{1}{1 + q/m_v}\right) \tag{8}$$

Then we set the adjusting and variable levers equal to each other, as in Equation 3:

$$\frac{1}{1 + q/m_a} = \sum_{v=1}^{n} p_v \left(\frac{1}{1 + q/m_v}\right) \tag{9}$$

This can be simplified in the same fashion as Equation 4, yielding the general equation for sensitivity to magnitude and adjusting magnitude:

$$\frac{1}{1+q/m_a} = (0.5)\left(\frac{1}{1+q/m_1}\right) + (0.5)\left(\frac{1}{1+q/m_2}\right) \qquad (10)$$

Finally, we can introduce the parameters used in the adjusting magnitude task in Chapter 2 (i.e. 15 µl and 135 µl for the variable lever), similar to Equation 5:

$$\frac{1}{1+q/m_a} = (0.5)\left(\frac{1}{1+q/15}\right) + (0.5)\left(\frac{1}{1+q/135}\right) \qquad (11)$$

Then we can solve for the relationship between adjusting magnitude (m_a) and sensitivity to magnitude (q) in a similar manner to that used for delay sensitivity:

$$m_a = \frac{75q + 2025}{q + 75} \qquad (12)$$

Within the constraints of $q \geq 0$ and $m_a \geq 0$, a decrease in q will result in a decrease in m_a (see Fig. 13b). Therefore, according to this equation, an increase in sensitivity to magnitude will result in an increase in adjusting magnitude.

The second model is derived from Killeen's (2009) additive-utility model of discounting. The sensitivity to magnitude aspect is rooted in marginal utility, which has a long history (e.g. Bernoulli, 1738/1954). Killeen's model differs from Ho et al.'s in that Ho et al.'s model represents sensitivity to either the discriminative properties of the

magnitude or sensitivity to the subjective value of the reward (≈ utility); the model does not distinguish the two (Ho et al., 1999). Killeen's model, on the other hand, only represents sensitivity to the subjective value of the reward (≈ utility; Killeen, 2009). Killeen's model is as follows:

$$U = m^{\alpha} \tag{13}$$

In this model, *m* represents a given objective value, α represents the individual's rate of diminishing marginal utility, and *U* is the subjective utility of that objective value.

Equation 13 can follow the same process that Equation 1 went through for delay. First, we modify the equation for the variable reinforcement contingency (as with Equation 2):

$$V_{m'} = \sum_{v=1}^{n} p_v({m_v}^{\alpha}) \tag{14}$$

Then we set the adjusting and variable levers equal to each other, as in Equation 3:

$${m_a}^{\alpha} = \sum_{v=1}^{n} p_v({m_v}^{\alpha}) \tag{15}$$

This can be simplified in the same fashion as Equation 4, yielding the general equation for sensitivity to magnitude and adjusting magnitude:

$$m_a{}^{\alpha} = (0.5)(m_1{}^{\alpha}) + (0.5)(m_2{}^{\alpha}) \qquad (16)$$

Finally, we can introduce the parameters used in the adjusting magnitude task in Chapter 2 (i.e. 15 µl and 135 µl for the variable lever), similar to Equation 5:

$$m_a{}^{\alpha} = (0.5)(15^{\alpha}) + (0.5)(135^{\alpha}) \qquad (17)$$

This can be solved for the relationship between m_a and α:

$$m_a = \left(\frac{15^{\alpha} + 135^{\alpha}}{2}\right)^{\left(1/\alpha\right)} \qquad (19)$$

Within the constraints of $\alpha \geq 0$ and $m_a \geq 0$, a decrease in α will result in a decrease in m_a (see Fig. 13c). Therefore, according to this equation, a decrease in sensitivity to magnitude will result in a decrease in adjusting magnitude.

It should be noted that Equations 7 and 13 have different predictions if incorporated into delay discounting equations. Modification of q in Equation 7 solely affects the intercept of the discounting function when used with Ho et al.'s (1999) multiplicative hyperbolic discounting function (see Fig. 1c). Contrarily, modification of α in Equation 13 has no effect on the intercept of the discounting function, but like k, alters the slope of the function when used within Killeen's (2009) additive-utility model of delay discounting (see Fig. 1b).

Lastly, it should be noted that there has been little empirical work regarding Equations 10 and 16. While their adjusting delay counterpart, Equation 4, has empirical

support (Mazur, 1984), the adjusting magnitude equations have not been formally investigated and have been taken as extrapolations of Equation 4. Nonetheless, data from da Costa Araújo et al. (2010) and the current work seem to show stronger support for Equation 16.

Appendix 2. Additional experiments associated with Chapter 2.

Effect of methamphetamine on the adjusting tasks

Introduction.

This study was conducted 1 week after the final experiment in Chapter 2 was completed. In that study, ethanol was not affected adjusting delay or adjusting magnitude, raising the possibility that the tasks were not sufficiently sensitive to reveal ethanol's effects. One way to address this issue is to examine whether other drugs would have an effect on task behavior. Methamphetamine was selected because a prior study has shown that acute methamphetamine decreased delay discounting in rats (Richards et al., 1999). Because decreased delay discounting would suggest either a decrease in sensitivity to delay or an increase in sensitivity to magnitude (see Appendix 1), it was anticipated that methamphetamine would either increase adjusting delay, increase adjusting magnitude, or do both.

Methods.

Subjects.

The same animals were used in this study as used in Chapter 2. One animal from the adjusting magnitude group died part way through this study and therefore was not included in the data analyses (adjusting delay n = 9, adjusting magnitude n = 8). Task assignments from the experiment described in Chapter 2 were preserved in this study. Animals were maintained on food restriction as in Chapter 2.

Apparatus

The same chambers were used in this study as used in Chapter 2.

Drugs.

Methamphetamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% saline and administered i.p. (0.0, 1.0, and 2.0 mg/kg), order counterbalanced. Each dose was administered once, for a total of 3 injections per rat. Injections were performed on Tuesdays and Fridays, and animals were placed in the task immediately following injections.

Procedure.

The tasks were identical to those used in Chapter 2.

Data Analysis.

Data analysis was identical to that described in Chapter 2. The primary dependent variable was the mean adjusting delay or magnitude value for the latter half of each session (last 45 trials). Date were analyzed using a 3 × 2 ANOVA (Dose × Injection; the "Injection" variable compared data from the injection days with data from the days immediately prior to the injection days to determine if there were any effects of injection itself). An additional series of 3 × 2 × 18 ANOVAs were performed to examine the effect of methamphetamine on the number of trials completed as a function of dose, injection and time during the session (number of trials completed was averaged into eighteen 5-min bins). Finally, a linear regression model using the adjusting delay or magnitude values averaged for the 10 days prior to ethanol exposure (same values as used in Chapter 2) was performed to predict the number of trials completed after methamphetamine administration for each dose. Number of trials completed on the pre-injection day was also used as a predictor variable in this model to control for differences

in baseline responding in the task. Unlike Chapter 2, the first 60 min rather than the first 20 min was used due to methamphetamine's sustained effect, although use of either time period yielded the same results.

Results.

There were no effects of methamphetamine on adjusting delay or adjusting magnitude at any dose (dose x injection, adjusting delay: $F(2,16) = .50$, $p = .614$; dose x injection, adjusting magnitude: $F(2,14) = .24$, $p = .787$; see Fig. 14). However, methamphetamine did dose-dependently decrease the rate at which trials were completed throughout the session, followed by a compensatory increase late in the session as shown by the Dose x Injection x Time bin analyses, although this failed to reach significance for the adjusting magnitude task (adjusting delay: $F(34,272) = 4.47$, $p < .001$; adjusting magnitude: $F(34,238) = 1.27$, $p = .156$; see Fig. 15). Examining the saline, 1.0 and 2.0 mg/kg dose conditions separately revealed that this effect was primarily driven by the 2.0 mg/kg dose (Saline, $F(17,136) = 1.07$, $p = .394$; 1.0 mg/kg, $F(17,136) = 1.35$, $p = .173$; 2.0 mg/kg, $F(17,136) = 6.81$, $p < .001$). However, after Bonferroni correction only the 70-75 min time bin showed a significant effect of methamphetamine. Neither adjusting delay nor adjusting magnitude predicted the decrease in number of trials completed (adjusting delay: $t = 0.40$, $p = .700$, partial $r = .163$; adjusting magnitude: $t = 0.80$, $p = .463$, partial $r = .335$; see Fig. 16).

Discussion.

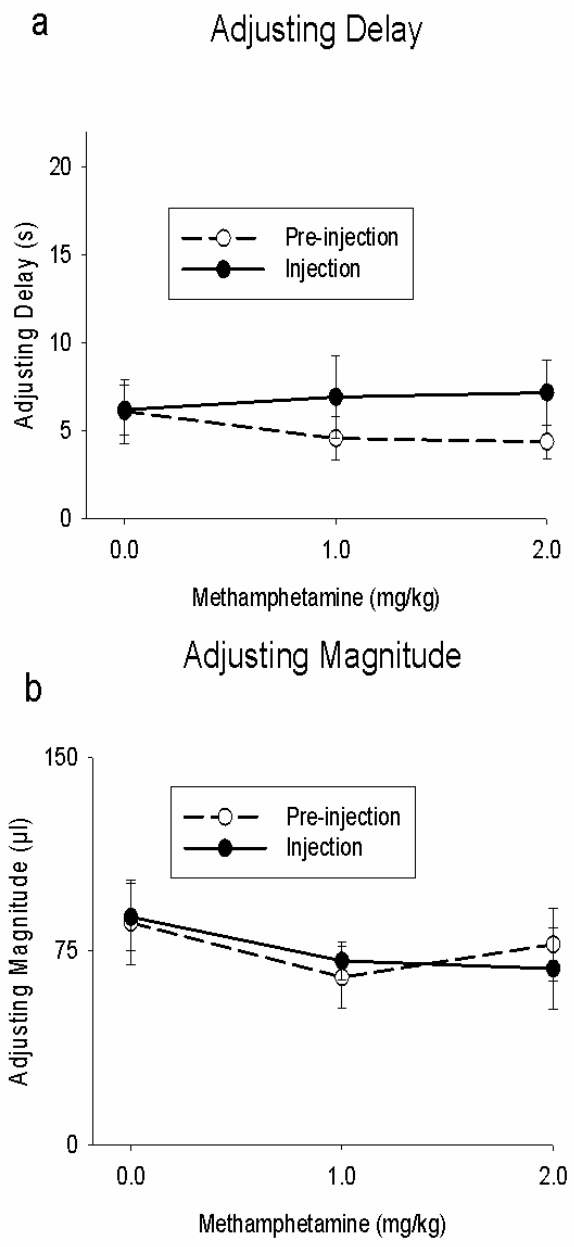

Figure 14. Methamphetamine did not affect adjusting delay **(a)** or adjusting magnitude **(b)**.

Methamphetamine had no effects on adjusting delay or adjusting magnitude, contrary to my expectations that were driven by the findings in Richards et al (1999). As was discussed in Chapters 2 and 3, the discrepancy between the Richards et al. data and those provided in this dissertation could be due to procedural differences. However, it should be noted that the study by Richards et al. (1999) used an adjusting procedure, so it is unlikely that the adjusting nature of the tasks used in this study were sufficient to obscure an effect. It is also possible that the null results are due to the differential nature of measuring sensitivity to delay and magnitude together versus in isolation.

Although methamphetamine had no effect on adjusting values, it did decrease the number of trials completed in the task, much like ethanol did in Chapter 2. However, unlike ethanol, neither adjusting delay nor adjusting magnitude predicted methamphetamine's effect on number of trials completed. This may be because methamphetamine is affecting operant responding via another mechanism. For example, 2.0 mg/kg methamphetamine increases locomotor activity in rats (Lucot et al., 1980), an effect not seen with 0.9 g/kg ethanol (the dose used in Chapter 2). Additionally, the doses of methamphetamine used only led to mild suppression in the number of trials completed, so it may be that a higher dose would have been required to see a relationship.

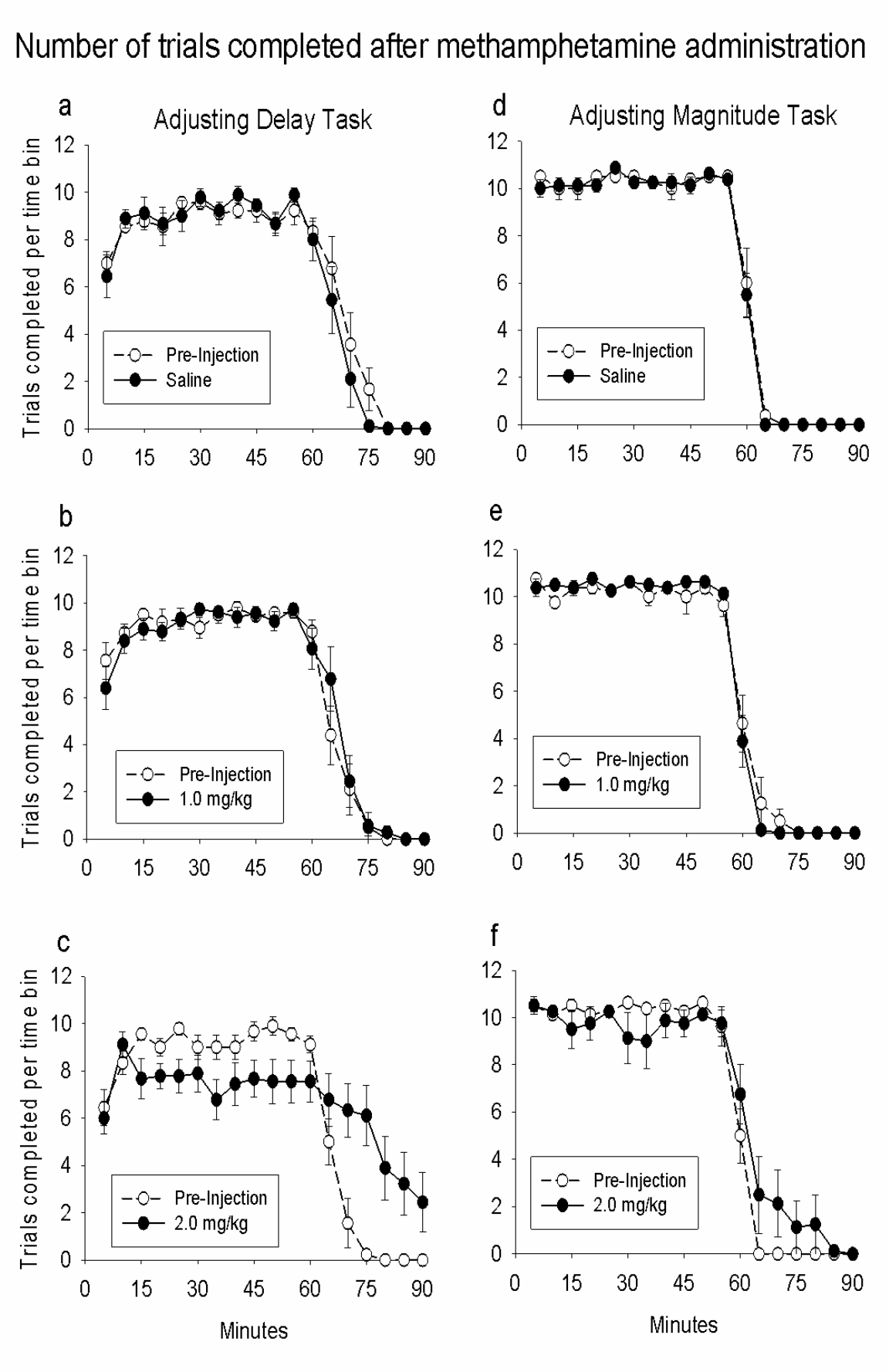
Number of trials completed after methamphetamine administration
a
Adjusting Delay Task
d
Adjusting Magnitude Task
b
e
c
f
Trials completed per time bin
Pre-Injection
Saline
1.0 mg/kg
2.0 mg/kg
Minutes
0
15
30
45
60
75
90
2
4
6
8
10
12

Figure 15. Methamphetamine (2 mg/kg) decreased the number of trials completed per time bin in both tasks, although this only reached significance in the adjusting delay task (**c, f**). On the days before methamphetamine injection, animals typically finished the task within 75 min (dashed lines). No effects were seen for the lower doses (**a**, **b**; **d**, **e**).

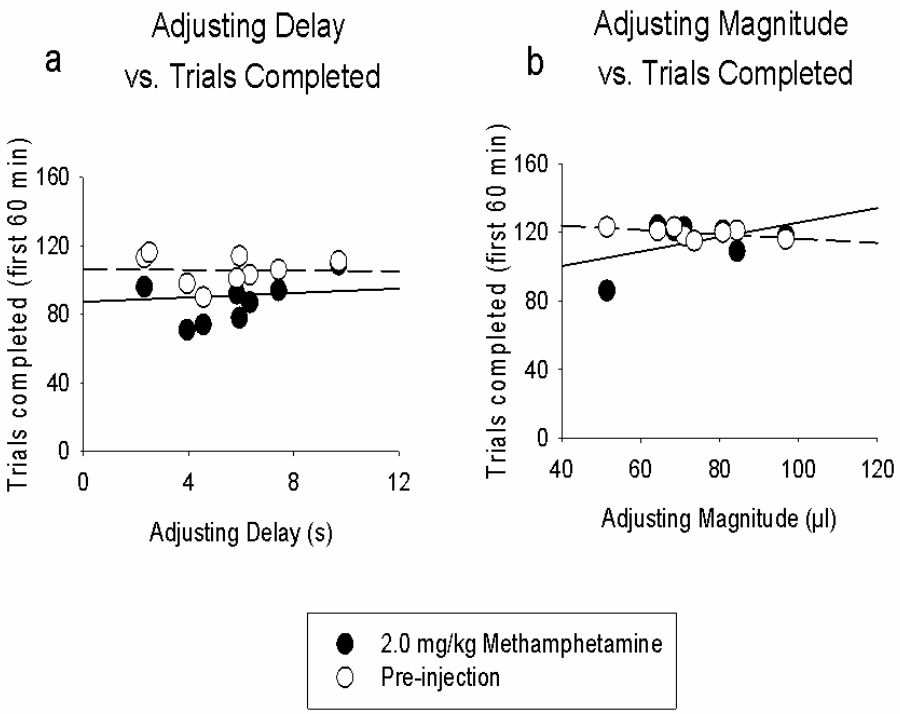

Figure 16. Relationship between average adjusting value and number of trials completed in the first 60 min after 2.0 mg/kg methamphetamine. There was no relationship between sensitivity to delay **(a)** or sensitivity to magnitude **(b)** and methamphetamine's behaviorally suppressant effects.

Effect of ethanol on locomotor activity

Introduction.

This study was conducted 2 days after completing the aforementioned methamphetamine study. In Chapter 2, 0.9 g/kg had a strong suppressant effect on operant behavior; an effect that was not seen in many other delay discounting studies using this or similar doses (Evenden & Ryan, 1999; Hellemans et al., 2005; Tomie et al., 1998). Ethanol has not been shown to affect horizontal locomotor activity at doses near 0.9 g/kg (e.g. 1.0 g/kg: Scott et al., 1994; 0.8 g/kg: Duncan et al., 2000), but it has been shown to effect vertical activity (0.8 g/kg: Duncan et al., 2000). The distinction between vertical and horizontal activity may be relevant to this dissertation because the levers used in Chapters 2 and 3 were placed 8 cm higher than is typically done in standard Med Associates boxes. Thus, this study was conducted to determine if 0.9 g/kg ethanol affected horizontal and/or vertical locomotor activity in the animals used in Chapter 2. Additionally, this experiment investigated if adjusting delay would predict resistance to any suppressant effects of ethanol on locomotor activity, similar to its ability to predict resistance to the suppressant effects of ethanol on operant activity.

Methods.

Subjects

The same animals that were used in Chapter 2 and the aforementioned methamphetamine experiment were used in this study, although one animal from the adjusting delay group died part way through the study and therefore was not included

(adjusting delay n = 8, adjusting magnitude n = 8). All animals were maintained on food restriction.

Apparatus

Five locomotor chambers (40 x 40 x 30 cm; Accuscan Instruments Inc., Columbus, OH, USA) housed in sound-attenuating cabinets were used in this study. Each chamber was equipped with 16 evenly spaced photocells located 2 cm above the floor and 8 evenly spaced photocells located 21 cm above the floor. The chambers were illuminated with a house light throughout the session and a fan provided ventilation and white noise. VersaMax version 4.12-125e software (Accuscan Instruments Inc., Columbus, OH, USA) was used to convert beam breaks into distance traveled, both vertically and horizontally (cm).

Procedure

All animals were given two habituation days in the chambers before beginning the injection phase of the experiment. Each day, rats were habituated to the locomotor room for 30 min prior to entering the locomotor chambers. Animals retained the same order of entry into the locomotor chambers as they had for the operant chambers. Locomotor activity was measured for 30 min, the approximate duration of the suppressant effects of 0.9 g/kg on operant behavior in Chapter 2.

Drugs

The doses of 0.0 and 0.9 g/kg ethanol were administered in the same fashion as in Chapter 2. Each dose was administered 3 times (6 injections total) on Tuesdays and Fridays in such a way that each dose was administered on the Tuesday or Friday of each week, and animals were placed in the locomotor chambers immediately after injection.

Data Analysis

"Task" (adjusting delay or adjusting magnitude) was included as a between subjects factor in the analyses, to examine whether the prior experimental experience of animals affected locomotor activity. Data were analyzed with a 2 x 2 x 2 x 3 ANOVA (Task x Dose x Injection x Occasion; the "Injection" variable compared data from the injection days with data from the days immediately prior to the injection days to determine if there were any effects of injection itself). Analyses examined both horizontal and vertical activity.

A linear regression model using either the adjusting delay lengths or magnitude values for the last 45 trials, averaged for the 10 days prior to any ethanol exposure (same values as used in Chapter 2) was performed to determine whether prior task performance could predict horizontal and vertical activity after ethanol administration. Activity on the pre-injection day was also used as a predictor variable in this model to control for differences in baseline levels of activity.

Results.

Ethanol had no effect on horizontal activity (Dose x Injection: $F(1,14) = 1.97$, $p = .182$) when collapsed across occasion, although it did decrease horizontal activity on the second occasion of administration (Dose x Injection x Occasion: $F(2,30) = 4.28$, $p = .023$; significantly different from saline on the second occasion only). It seems unlikely that the lack of effect on the other occasions is due to tolerance because administration of ethanol on the first occasion had no effect. In contrast to horizontal activity, ethanol significantly decreased vertical activity (Dose x Injection: $F(1,14) = 10.27$, $p = .006$, significant differences between saline and 0.9 g/kg after Bonferroni correction; see Fig.

17). Neither adjusting delay or adjusting magnitude predicted any effect of ethanol on either horizontal or vertical activity (ts < 2.31, ps > .069, partial rs < .719; see Fig. 18).

Discussion.

Ethanol (0.9 g/kg) decreased vertical activity, but had no effect on horizontal activity. This fits with previous work showing that ethanol impairs vertical activity at lower doses than it does horizontal activity in rats (Duncan et al., 2000). The current finding suggests that the ethanol's abnormally strong behaviorally suppressant effects in the adjusting tasks were likely the result of a deficit in the ability and/or motivation to rear. This idea is reinforced because the levers used in the operant chambers in Chapter 2 were placed at a height that required the rats to rear in order to press them (11 cm above the grid floor). Presumably, the use of lower levers would have greatly ameliorated the suppressant effects of ethanol.

Although adjusting delay length predicted the suppressant effects of ethanol on operant behavior in Chapter 2, it did not predict the suppressant effects of ethanol on locomotor activity. This is a bit surprising, because ethanol suppresses both operant responding and vertical activity, but adjusting delay only predicts the former. This may suggest that ethanol has a composite effect on both vertical activity and some other process, such as motivation to respond in the task, and that adjusting delay only predicts its effect on the latter process. Alternatively, it is possible that ethanol only affects vertical activity, and that adjusting delay predicts motivation to respond in the task regardless of ethanol administration. In this scenario, the high motivation of animals with low adjusting delay would allow them to overcome the suppressant effects of

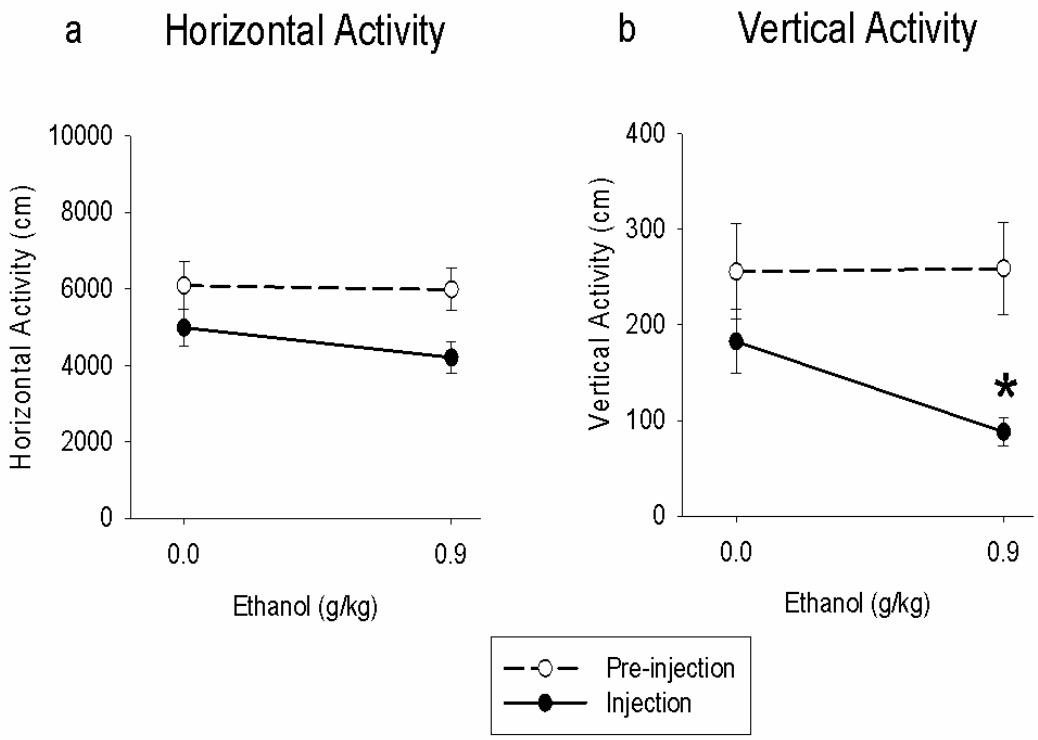

Figure 17. Ethanol (0.9 g/kg) had no effect on horizontal activity **(a)**, but decreased vertical activity **(b)**.

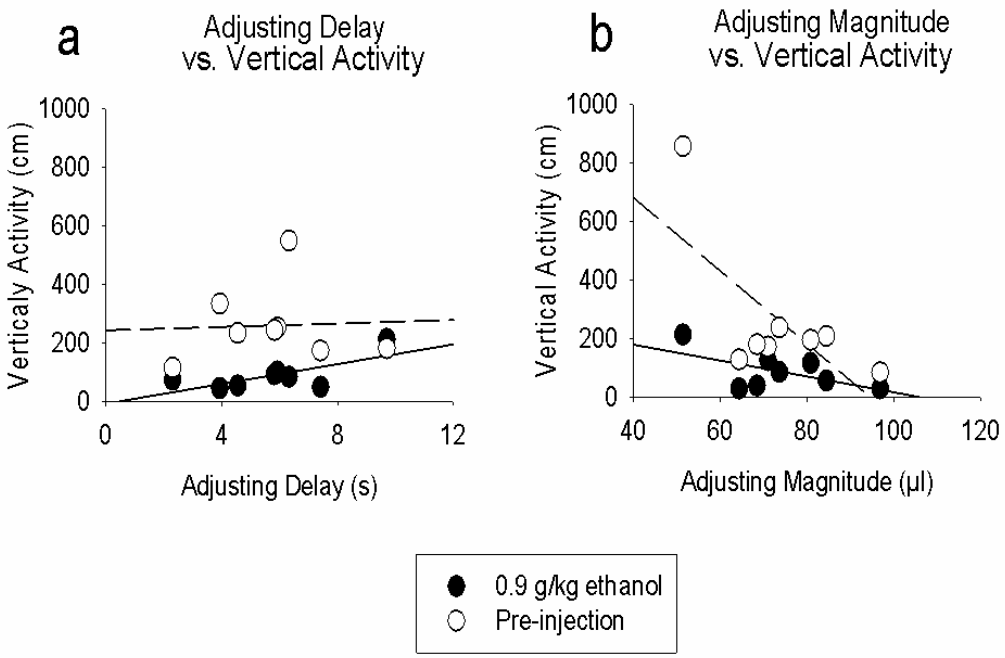

Figure 18. Relationship between average adjusting value and vertical activity in the locomotor chamber. There was no relationship between sensitivity to delay **(a)** or sensitivity to magnitude **(b)** and ethanol's behaviorally suppressant effects.

ethanol on vertical activity. If this were the case, however, one might expect low adjusting delay to predict faster reaction times, which was not the case. Nonetheless, it may be more appropriate to administer a different measure, such as a progressive ratio task, to truly assess motivation in these animals. At any rate, while these are intriguing and testable models, it is important to note that these animals had several doses of ethanol and methamphetamine before beginning this experiment, and that such a history requires a cautious interpretation of these results and verification with initially drug naïve animals.

Appendix 3. Additional experiments associated with Chapter 3.

Effect of lOFC and AcbC inactivation on locomotor activity

Introduction.

As described in Chapter 3, inactivation of the lOFC decreased the number of trials completed for all tasks. Additionally, doses of AcbC baclofen/muscimol, which had minimal effects on operant responding in other studies (McFarland & Kalivas, 2001; Stopper & Floresco, 2011), strongly suppressed responding in the tasks used in Chapter 3. Thus, this study was initiated immediately after completion of the study described in Chapter 3 to determine if these suppressant effects on operant activity were the result of a locomotor deficit.

Methods.

Subjects

The same animals were used in this study as used in Chapter 3. All animals were maintained on food restriction throughout. While all subjects from Chapter 3 participated, data from animals subsequently demonstrated to have poor placement were excluded from all analyses.

Apparatus

The locomotor chambers were the same as those used in Appendix 2.

Procedure

All animals were given 1 habituation day in the locomotor chambers before beginning the microinjection procedure (which included an additional pre-injection day

followed by the injection day; 3 days total). Immediately after microinjection of baclofen/muscimol or saline, animals were transported to the room containing the locomotor chambers to habituate to the room (the locomotor room was less than 10 m away from the infusion/operant room). Animals were then given 10 min to allow them time to recover from the stress of injection. Subsequently, they were placed in their respective locomotor chambers for 60 min, because the suppressant effects of inactivation lasted the entire 60 min duration of the operant tasks used in Chapter 3. Animals were placed in boxes in the same order as they had been placed in the operant chambers used in Chapter 3.

Drugs

lOFC animals received bilateral 0 ng / 0 ng or 125 ng / 125 ng baclofen/muscimol. AcbC animals received bilateral 0 ng / 0 ng, 6.409 ng / 0.342 ng, 64.09 ng / 3.42 ng, or 75 ng / 75 ng baclofen/muscimol. The 6.409 ng / 0.342 ng AcbC dose was that used in Chapter 3, while the two higher doses were those used by McFarland & Kalivas (2001) and Stopper & Floresco (2011), but found to have too large of a suppressant effect to use in the experiment detailed in Chapter 3. To minimize further damage to the brain, each rat only received 1 microinjection. Animals were assigned randomly to each dose group. Final group sizes were: AcbC Saline n = 8, AcbC 6.409 ng / 0.342 ng n = 8, AcbC 64.09 ng / 3.42 ng n = 7, AcbC 75 ng / 75 ng n = 7, lOFC Saline n = 16, lOFC 125 ng / 125 ng n = 15.

Data Analysis

Because each animal only received 1 microinjection, dose was a between subjects factor. Injection remained a within subjects factor as in previous studies. Either a 2 x 2

(lOFC) or 4 x 2 (AcbC) ANOVA (Dose x Injection) was used to analyze horizontal and vertical activity. When discussing the results, "Injection" data refers to data collected on the day that the animal received an injection, while "Pre-injection" data refers to data collected on the day immediately prior to the injection day (note that the pre-injection day is the day after the habituation day). Because there were large differences between AcbC dose groups on pre-injection days (see Fig. 19; in hindsight it would have been better to match animals rather than use random assignment), it would be inappropriate to conduct further analyses without including the pre-injection data. Thus, I used a 2 x 2 ANOVA (Dose x Injection) to determine the effects of each dose individually compared to saline.

Results.

lOFC inactivation had no effect on either horizontal or vertical activity (dose x injection: horizontal: $F(1,29) = .07$, $p = .793$; vertical: $F(1,29) = .494$, $p = .488$; see Fig. 19a, b).

AcbC inactivation dose-dependently decreased both horizontal and vertical activity (dose x injection: horizontal: $F(3,26) = 4.65$, $p = .010$; vertical: $F(3,26) = 4.40$, $p = .013$; see Fig. 19c, d). Further analysis suggested that the effect on horizontal activity was entirely driven by the 75 ng / 75 ng dose (dose x injection: 75 ng / 75 ng: $F(1,13) = 6.52$, $p = .024$; all other $Fs < .14$, $ps > .715$). Contrarily, every dose appeared to decrease vertical activity when compared to saline, although the 64.09 ng / 3.42 ng dose only exhibited a trend (dose x injection: 6.409 ng / 0.342 ng: $F(1,14) = 4.95$, $p = .043$; 64.09 ng / 3.42 ng: $F(1,13) = 3.27$, $p = .094$; 75 ng / 75 ng: $F(1,13) = 9.75$, $p = .008$).

Discussion.

The effects of inactivation on locomotor activity varied according to dose and brain region. Inactivation of the lOFC did not affect locomotor activity. This suggests that the suppression in operant task responding was unlikely to be due to a deficit in locomotor activity. The fact that lOFC inactivation also increased the number of extraneous lever press the rats made strengthens the conclusion that lOFC inactivation was altering some other process.

The highest dose of baclofen/muscimol in the AcbC greatly suppressed both horizontal and vertical activity, while the lower doses appeared to only decrease vertical activity. However, the latter effect is difficult to interpret due to the large variation between subjects in their drug-free locomotor activity. These results suggest that the strong suppressant effects of the two highest doses of AcbC baclofen/muscimol in the operant tasks may have been partially due their effect on the ability/motivation to rear. This idea is given further credence by the fact that the levers in the operant tasks used in Chapter 3 were 8 cm higher than those used in both McFarland & Kalivas (2001) and Stopper & Floresco (2011), which had found minimal effects of the two highest doses on operant activity. The veracity of this suggestion could be tested by lowering the height of the operant chamber levers, and determining if the behaviorally suppressant effects of AcbC inactivation were ameliorated. Such a test was conducted in the second study listed in this Appendix.

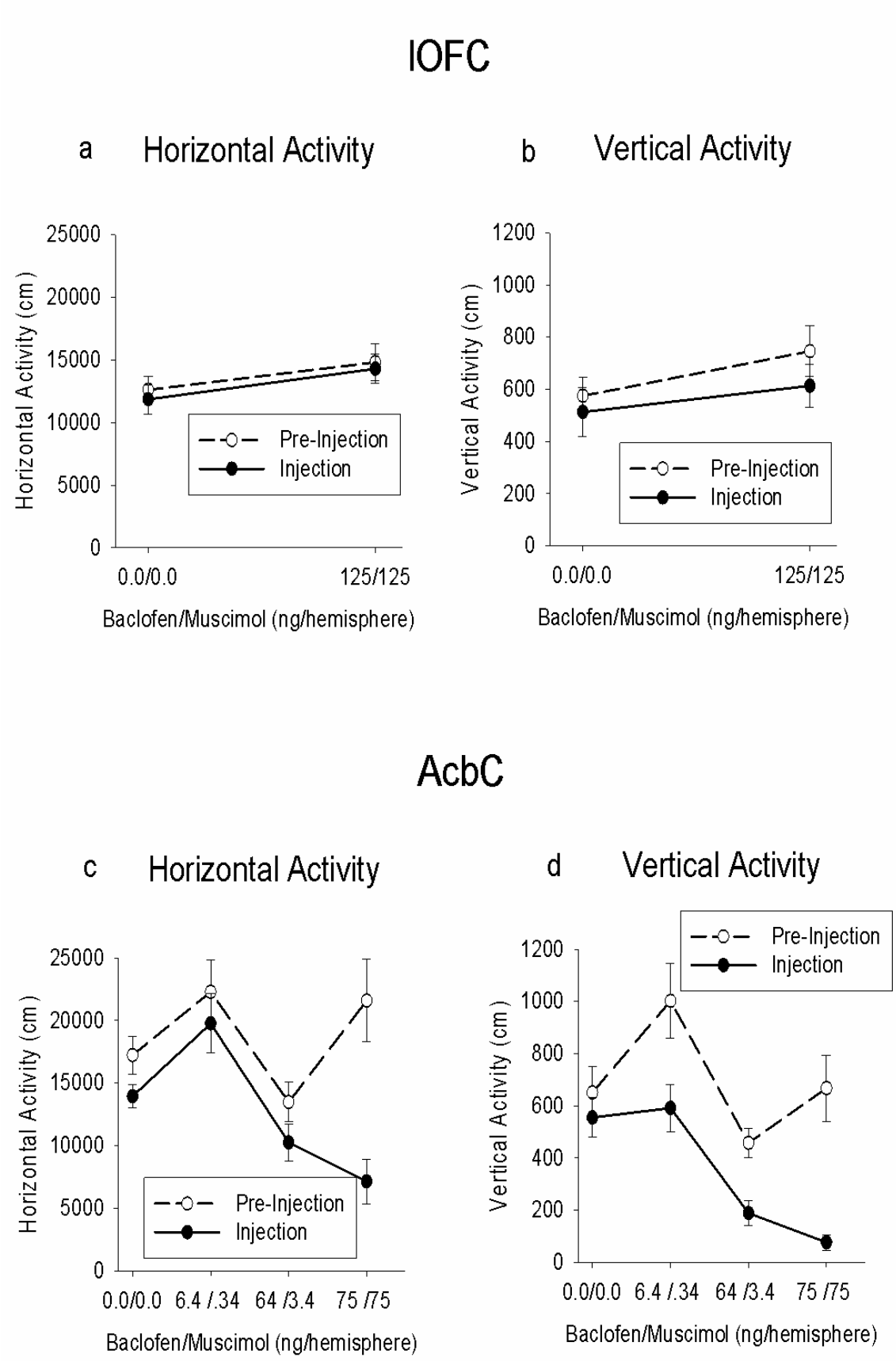
lOFC
a
Horizontal Activity
Horizontal Activity (cm)
25000
20000
15000
10000
5000
0
Pre-Injection
Injection
0.0/0.0
125/125
Baclofen/Muscimol (ng/hemisphere)
b
Vertical Activity
Vertical Activity (cm)
1200
1000
800
600
400
200
0
Pre-Injection
Injection
0.0/0.0
125/125
Baclofen/Muscimol (ng/hemisphere)
AcbC
c
Horizontal Activity
Horizontal Activity (cm)
25000
20000
15000
10000
5000
0
Pre-Injection
Injection
0.0/0.0
6.4 /.34
64 /3.4
75 /75
Baclofen/Muscimol (ng/hemisphere)
d
Vertical Activity
Vertical Activity (cm)
1200
1000
800
600
400
200
0
Pre-Injection
Injection
0.0/0.0
6.4 /.34
64 /3.4
75 /75
Baclofen/Muscimol (ng/hemisphere)

Figure 19. Effect of inactivation on locomotor behavior. Inactivation of the lOFC had no effect on horizontal and vertical activity (**a**, **b**). Inactivation of the AcbC dose-dependently affected locomotor activity. Only the 75 ng / 75 ng dose significantly decreased horizontal activity when taking into account pre-injection activity levels (**c**). However, all doses beyond saline significantly decreased vertical activity when taking into account pre-injection activity levels (**d**).

Effects of lOFC and AcbC inactivation on operant behavior: Role of lever height

Introduction.

In the first study in this Appendix, AcbC inactivation decreased vertical activity at lower doses than it affected horizontal activity, while lOFC activity had no effect on either. These results suggest that the strong suppressant effects of the two highest doses of AcbC baclofen/muscimol in the operant tasks may have been partially due their effect on the ability/motivation to rear. This idea is given further credence by the fact that the levers in the operant tasks used in Chapter 3 were 8 cm higher than those used in both McFarland & Kalivas (2001) and Stopper & Floresco (2011), which had found minimal effects of the two highest doses on operant activity. To determine if lever height was playing role in the suppressant effects of AcbC inactivation, the height of the levers in the operant chambers were lowered in this study. I hypothesized that this would ameliorate the suppressant effects of AcbC inactivation, but have no effect on the suppressant effects of lOFC inactivation. This study was conducted immediately following the completion of the first study mentioned in this study.

Methods.

Subjects

Animals from the first cohort in Chapter 3 were used in this study (n = 43; 5 died before reaching this study). While all remaining subjects from the first cohort participated, data from animals subsequently demonstrated to have poor placement were excluded from all analyses.

Apparatus

Four of the operant chambers used in Chapter 3 had the following modifications. The two levers were lowered from 11 cm to 3 cm above the grid floor. This necessitated the removal of the two sucrose receptacles that were directly below the levers. To replace them, the center nosepoke was removed and one sucrose receptacle was placed there. Thus, response on either lever would result in the appropriate amount of sucrose delivered into the center receptacle. The remaining four chambers retained 11 cm high levers, but the center nosepoke was replaced with a sucrose receptacle, and the other sucrose receptacles were replaced with steel plates. Therefore, the "high lever" chambers were identical in every way to the "low lever" chambers except in lever height. All animals were assigned to the same chambers they had previously been in for Chapter 3. Twenty-one animals were assigned to "low lever" chambers, while 22 were in "high lever" chambers.

Procedures

All animals were run in their respective tasks (see Fig. 8) from Chapter 3, modified for the newly-altered chambers. The only difference from the old tasks was that there was no requirement to make the center nosepoke to start the trial, since the center nosepoke had been removed.

Drugs

lOFC animals received 125 ng / 125 ng baclofen/muscimol. AcbC animals received 64.09 ng / 3.42 ng and 75 ng / 75 ng baclofen/muscimol. All lOFC animals received one injection. Sixteen AcbC animals received two injections (one each of 75 ng / 75 ng and 64.09 ng / 3.42 ng) while 3 only received one injection (75 ng / 75 ng) because they were unable to learn the task by the first injection day. Saline was not used because Chapter 3 had already demonstrated that saline had no effect on number of trials

completed in the original boxes that had high levers and in order to minimize brain damage.

Data analysis

Number of trials completed was analyzed with independent t-tests for each dose, comparing the low lever animals to the high lever animals.

Exclusions

Final group sizes were: AcbC high lever n = 8, AcbC low lever n = 9, lOFC high lever n = 6, lOFC low lever n = 7. One animal received the wrong injection, and 5 animals were unable to learn the new (lower lever height) task.

Results.

Low lever animals completed more trials than high lever animals after both AcbC doses (64.09 ng / 3.42 ng: $t(14) = 2.55$, $p = .023$; 75 ng / 75 ng: $t(15) = 3.63$, $p = .003$; see Fig. 20). No effect was seen with lOFC inactivation ($t(11) = 0.17$, $p = .866$).

Discussion.

Lowering the height of the levers greatly ameliorated the behaviorally suppressant effects of AcbC inactivation. This finding fits well with the locomotor study, which showed that AcbC inactivation decreased vertical activity at lower doses than it decreased horizontal activity. Additionally, this finding offers the most likely explanation as to why these doses suppressed behavior in the tasks used in my study while having much smaller effects in other studies which used lower levers (Stopper & Floresco, 2011; McFarland & Kalivas, 2001). However, it should be noted that some animals had difficulty in learning the new task and that external time constraints did not allow animals enough time to

attain stability in the new task. An appropriate replication of this study would require a greater span of time during which animals could attain stable responding in the task.

Lowering the height of the levers had no effect on the suppressant effects of lOFC inactivation. This finding also fits well with the locomotor study, which suggested that lOFC inactivation had no effect on locomotor activity. Thus, the behaviorally suppressant effects of lOFC inactivation are likely through some other mechanism. The implications of these findings are discussed further in the General Discussion.

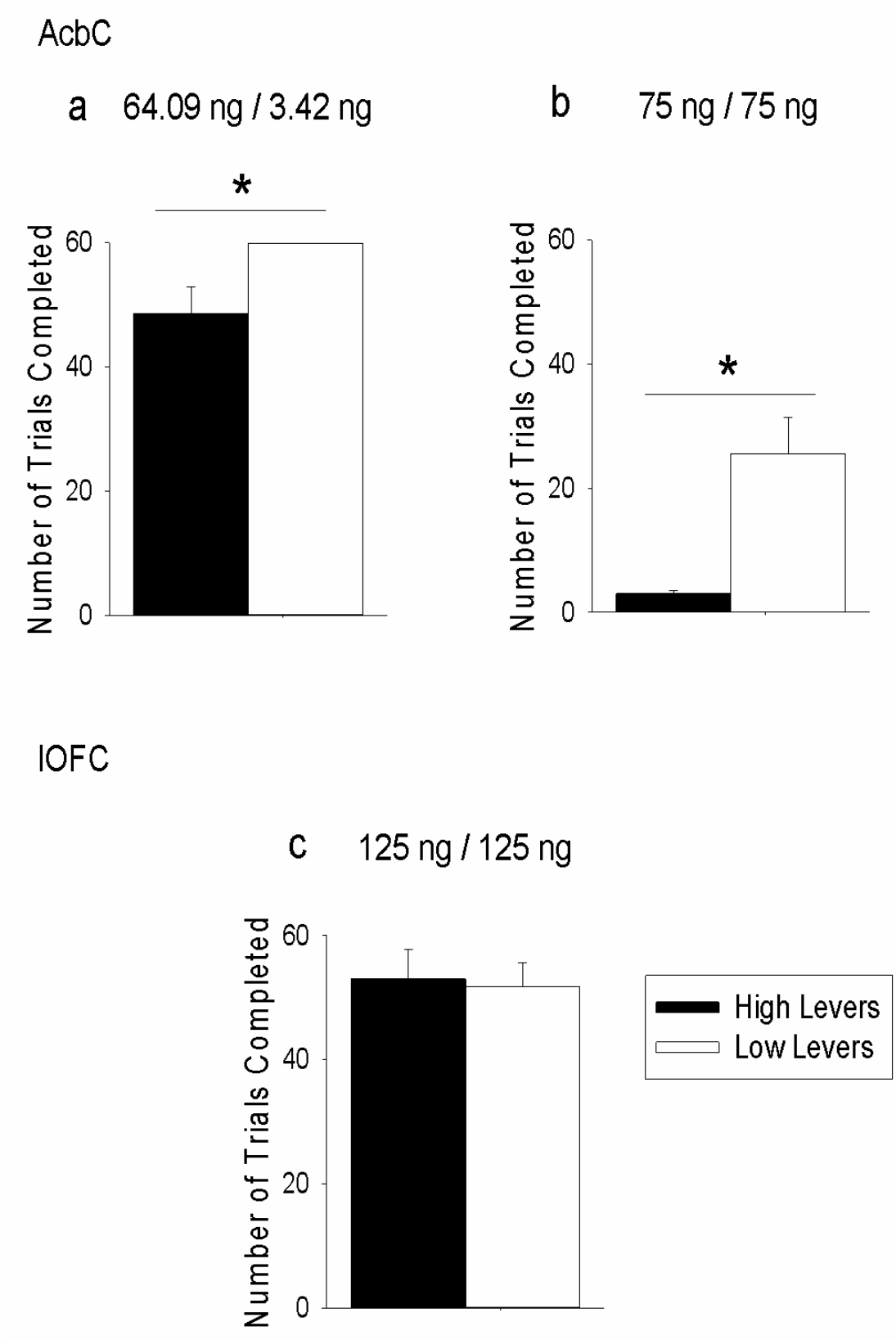
AcbC
a 64.09 ng / 3.42 ng
b 75 ng / 75 ng
*
*
Number of Trials Completed
60
40
20
0
IOFC
c 125 ng / 125 ng
High Levers
Low Levers

Figure 20. Effect of inactivation on number of trials completed in high lever and low lever chambers. Inactivation of the AcbC of animals in high lever chambers led to significantly fewer trials completed than inactivation of the AcbC of animals in low lever chambers (**a**, **b**). Inactivation of the lOFC led to similar numbers of trials completed in both high lever and low lever chambers (**c**). * $p < .05$